Major Advances in Fundamental Dairy Cattle Nutrition

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ABSTRACT

Fundamental nutrition seeks to describe the complex biochemical reactions involved in assimilation and processing of nutrients by various tissues and organs, and to quantify nutrient movement (flux) through those processes. Over the last 25 yr, considerable progress has been made in increasing our understanding of metabolism in dairy cattle. Major advances have been made at all levels of biological organization, including the whole animal, organ systems, tissues, cells, and molecules. At the whole-animal level, progress has been made in delineating metabolism during late pregnancy and the transition to lactation, as well as in whole-body use of energy-yielding substrates and amino acids for growth in young calves. An explosion of research using multicatheterization techniques has led to better quantitative descriptions of nutrient use by tissues of the portal-drained viscera (digestive tract, pancreas, and associated adipose tissues) and liver. Isolated tissue preparations have provided important information on the interrelationships among glucose, fatty acid, and amino acid metabolism in liver, adipose tissue, and mammary gland, as well as the regulation of these pathways during different physiological states. Finally, the last 25 yr has witnessed the birth of “molecular biology” approaches to understanding fundamental nutrition. Although measurements of mRNA abundance for proteins of interest already have provided new insights into regulation of metabolism, the next 25 yr will likely see remarkable advances as these techniques continue to be applied to problems of dairy cattle biology. Integration of the “omics” technologies (functional genomics, proteomics, and metabolomics) with measurements of tissue metabolism obtained by other methods is a particularly exciting prospect for the future. The result should be improved animal health and well being, more efficient dairy production, and better models to predict nutritional requirements and provide rations to meet those requirements.

Key words: metabolism, nutrition, molecular biology, splanchnic tissue

INTRODUCTION

Dairy cattle nutrition can be defined broadly as the use of the components of feeds for the processes of maintenance, growth, reproduction, lactation, and health. Applied nutrition is the selection and proportioning of feedstuffs and ingredients to supply the correct amounts and balance of nutrients required for optimal productive and reproductive performance. Fundamental nutrition is the series of biochemical reactions used in the body during the assimilation and processing of nutrients to meet the physiological needs of the animal. Fundamental and applied nutrition are equally important in determining optimal feeding and management strategies for dairy cattle for health and production. This review will mainly highlight aspects of basic or fundamental nutrition that are referred to as metabolism, which encompasses many disciplines including biochemistry, physiology, and molecular biology.

Twenty-five years ago, the nutrients required by dairy cattle were known and most of their biochemical functions had been established. Nutrient requirement and diet formulation systems, however, were still largely based on “black box” concepts such as net energy and crude protein. In the 75th anniversary issue of the *Journal of Dairy Science*, Paul Moe from the USDA Beltsville research station reviewed progress in energy metabolism research in dairy cattle over the preceding 25 yr. In summarizing, Moe suggested areas of dairy nutrition research for the next 25 yr that he believed would enable the formulation of diets for modern dairy cows that allowed them to achieve their maximal potential for milk production with minimal stresses. To formulate diets that promoted maximal intake and effective absorption and metabolism of nutrients, he emphasized the need for greater understanding of the quantitative relationships among diet, the end products of digestion, and animal performance, and specifically the effects of individual nutrients on milk component production and nutrient partitioning.

The ensuing 25-yr period has seen noteworthy progress in opening the black boxes of net energy and crude

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protein. One indication that our understanding of fundamental nutrition has advanced is the progression of concepts and models in the two editions of *Nutrient Requirements of Dairy Cattle* published by the National Research Council (NRC) Subcommittee on Dairy Cattle Nutrition during this period. The 1989 edition moved toward a more mechanistic approach with the establishment of undegraded intake protein and degraded intake protein, as well as providing equations and simple software. The 2001 version is a significant step forward, and has begun to move the field toward more mechanistic definition of nutrient requirements. In this edition, a dynamic model of nutrient requirements was developed that is based largely on fundamental knowledge of metabolism and physiology. Along with other models of dairy cattle nutrition (e.g., Cornell Net Carbohydrate and Protein System) and metabolism (e.g., the UC-Davis "Molly"), the NRC publication demonstrates that our quantitative knowledge of fundamental nutrition, and how to use that knowledge to better feed dairy cattle, has improved markedly during the last 25 yr.

In this article, our aim is to highlight areas where we believe major advances in understanding have been made toward the challenge put forward by Moe 25 yr ago, and that have led the field to the current state of knowledge. Many of these accomplishments have been made using traditional and classic nutritional or biochemical procedures; on the other hand, major advances have also been made possible by techniques that did not exist or were in their infancy 25 yr ago.

PROGRESS IN UNDERSTANDING WHOLE-ANIMAL METABOLISM

Over the last 25 yr, considerable progress has been made in aggregating knowledge from various biological levels (whole animal, organ system, tissues, cells, molecules) into a more complete picture of metabolism in the whole animal, as affected by its genetic background, physiological state, environment, and diet. The dairy production enterprise requires accurate models of nutrient metabolism for cattle at all stages of the life cycle, from birth to maturity. Although our knowledge is far from complete, we now have more useful mechanistic models to predict and evaluate performance of both lactating and growing cattle that incorporate an improved fundamental understanding of metabolism. In particular, the last 25 yr has seen significant progress in describing metabolism during several key life-cycle stages that were inadequately understood previously. A few key examples follow.

A number of research groups have tackled the difficult challenge of defining fundamental requirements for nutrients in cows during late pregnancy and into

early lactation. Previously, requirements for energy and protein for pregnancy had been established largely based on differences in calorimetric measurements and balance studies between pregnant and nonpregnant cows, with a few small and incomplete studies of conceptus growth. Bell and colleagues at Cornell University undertook a comprehensive slaughter study to quantify the accumulation of nutrients in the developing fetus and maternal reproductive tissues of Holstein cows at increasing days of pregnancy. Their data set included measurements of energy, protein, and minerals and provided quantitative estimates of the rates of accretion with advancing pregnancy. Through their studies, the nutrient requirements of pregnancy have been much more clearly defined, and that knowledge has been incorporated into newer nutritional models including NRC (2001).

A major area of emphasis and progress during the last quarter-century has been to understand the rapidly changing nutritional requirements and nutrient metabolism during the "transition period" from late gestation to early lactation. Before the 1980s, the transition period was largely ignored, and even avoided, by researchers because of the extreme variability in animal responses and the frequent incidence of health problems. Several research groups had been working on various aspects of dry period nutrition and metabolism before the 1980s. However, groundbreaking studies from the laboratory of Ric Grummer at the University of Wisconsin were instrumental in focusing research and field attention to the need to consider these cows as distinct physiological and metabolic entities. The Wisconsin studies established that declining DM intake (DMI) in the last few days before parturition was a major factor in setting into motion a series of events that included increased body fat mobilization, increased uptake of nonesterified fatty acids (NEFA) by liver, and accumulation of triacylglycerol (TG) in liver. Along with key studies dealing with nutritional maintenance of blood calcium by Elliott Block, Jesse Goff, Ron Horst, and Dave Beede, among others, these studies created awareness of the importance of the transition period both to dairy profitability and to animal well-being. Investigations into the biology of transition or periparturient cows has flourished across North America in several research groups (University of Alberta, Cornell University, University of Guelph, University of Illinois, Michigan State University, National Animal Disease Center, The Ohio State University, Purdue University, and the University of Wisconsin, among others) as well as other groups worldwide. Research over the last decade has begun to dissect the metabolic basis for adaptations in liver, adipose tissue, skeletal muscle, bone, and mammary gland during the transition period. Prog-

ress in understanding the unique physiology of the transition cow has led to improved understanding of nutrient requirements, although optimal nutrition and feeding practices are far from resolved.

Another life-cycle stage where significant progress has been made is in better defining nutrient metabolism and requirements in the preruminant calf. Over the last few years, researchers at Cornell University and the University of Illinois undertook a reevaluation of nutrients required for growth, for several reasons. First, the 1989 NRC publication did not adequately describe requirements for calves, and the specifications therein did not accurately predict growth in the field. Second, both the genetic makeup of modern calves and the ingredient makeup of their diets had undergone marked changes, as genetics led to leaner cattle and whey proteins replaced dried skim milk in milk replacers. Finally, the accelerating evolution of the dairy industry to larger herd size and the advent of custom calf-rearing enterprises contributed to increase attention on the calf and its fundamental biology.

Van Amburgh and colleagues at Cornell University aimed to extend their models for heifer growth (developed earlier) to the young preruminant calf. Illinois researchers sought to better define the relationships between supplies of digestible protein and energy. Both groups used a combination of balance trials and the comparative slaughter approach to determine changes in composition of visceral tissues and carcass during early growth. Measurements of the composition of body tissue at different rates of growth and as affected by dietary composition have confirmed many of the principles established by the 2001 NRC committee, and have provided a framework for subsequent improvements in requirement systems for energy and nitrogen in calves fed milk replacer. Moreover, this research demonstrated that body composition can be markedly affected by the ratio of protein to energy, and served as a reminder that conventional calf-rearing systems have limited nutrient intake and lean growth far below the inherent capacity of the animal. The improved description of the most fundamental concepts of growth—that is, the metabolic use of dietary protein and energy-yielding nutrients for deposition of body tissue—has already led to several practical systems to capitalize on the remarkable growth potential of the very young calf.

In addition to these advances in life-cycle metabolism, advances have been made in several other areas of fundamental nutrition. The most important variable in nutrient requirement and rationing models is DMI. Progress in understanding the regulation of DMI has occurred, both through whole-animal modeling approaches as well as more mechanistic studies of neural, endocrine, and metabolic influences. Several groups

have made fundamental advances in understanding rumen function and its role in provision of nutrients. Advances include a more complete understanding of the microbial population responsible for digestion of fibrous and nonfibrous carbohydrates and the synthesis of microbial protein, enhanced descriptive chemistry of feed components, and more robust models of how the rumen microbial population digests those components.

The preceding 25 yr has seen an explosion of knowledge in whole-animal metabolism of fatty acids. In the late 1970s, research on fat nutrition and use by ruminants surged, so that research from the 1980s to the present has resulted in a fairly complete picture of how dairy cattle digest, absorb, and metabolize fatty acids of dietary and endogenous origin. In the last 15 yr, research and understanding in this area has been greatly stimulated by the discovery by M. W. Pariza at the University of Wisconsin of the potent anticarcinogenic effects of conjugated linoleic acid (CLA), which is a group of isomers of linoleic acid produced in the rumen and in animal tissues. The *cis*-9, *trans*-11 isomer of CLA is responsible for the anticancer effects. Because the rumen microbial population is the source of CLA or its precursor, *trans* monounsaturated fatty acids, research has been refocused intensively on lipid nutrition and metabolism. Several research groups led by Dale Bauman at Cornell University, Rich Erdman and Bev Teter at the University of Maryland, and Joe Herbein at Virginia Tech University discovered the powerful suppressive effects of various microbially derived fatty acids having *trans*-10 double bonds on fat synthesis in mammary gland and adipose tissue. These findings, which were a by-product of the search for ways to enhance the content of the anticarcinogenic CLA isomers in milk and beef, confirmed the hypothesis of Carl Davis and Dick Brown in the late 1960s that *trans* fatty acids produced in the rumen of cows fed high-grain diets might be responsible for milk fat depression.

Advances have been made in whole-animal aspects of amino acid metabolism, ranging from prediction of intestinal supply to tissue metabolism to detailed mechanistic models published by large modeling teams. Work by several groups to unravel the tissue requirements for amino acids has continued and progressed, so that better estimates of amino acid requirements for milk production and other functions can now be made. This area likely will receive renewed research focus over the next 25 yr as demands for more efficient use of dietary nitrogen and less nitrogen excretion into the environment continue to intensify.

Finally, advancements have been made in fundamental nutrition of minerals and vitamins. A major achievement has been in understanding factors that control calcium mobilization from bone during times of in-

creased tissue calcium demand and dietary calcium insufficiency. In particular, the role of dietary cation-anion balance and vitamin D in regulating this process has led to fundamental changes in how cows are managed during the transition from dry period to lactation. A reexamination of phosphorus requirements and metabolism is moving the field toward lower dietary supply of P, thereby improving environmental outcomes. Several groups have revisited the metabolic role of certain B vitamins, such as biotin and folic acid, during the transition period and have provided evidence that the long-held view that ruminants need no supplemental B vitamins may be incorrect for certain key life-cycle stages. Significant progress in understanding the metabolic roles of trace minerals and vitamins, especially vitamin E and selenium, in immune processes are discussed elsewhere in this issue.

Much of the research described in this section has used traditional nutritional and biochemical tools, such as dose-response feeding trials, balance studies, intestinally cannulated animals, radioisotope tracer studies, and concentrations of metabolites and hormones in blood. Although these techniques have provided invaluable information, techniques that were not available or not perfected 25 yr ago have pushed back the boundaries of knowledge on organ systems and cellular regulation of metabolism.

PROGRESS IN UNDERSTANDING NUTRIENT ABSORPTION AND METABOLISM BY SPLANCHNIC TISSUES

Paul Moe contended 25 yr ago that a greater understanding of the quantitative relationships among diet, the end products of digestion, and animal performance, along with mathematical models describing the effects of diet on nutrient absorption and metabolism, were needed to improve feeding systems and to better predict responses to diet composition. This vision, shared by many ruminant nutritionists at the time, spurred a substantial body of research that quantified nutrient fluxes across the splanchnic tissues of ruminants and also fostered development of mathematical models of nutrient flow and metabolism in the dairy cow, such as the UC-Davis model “Molly” and other mechanistic models of nutrient metabolism. The emphasis on effects of nutrient supply on productive response reflected the belief shared by many dairy nutrition researchers that nutrient supply is a direct determinant of productive response. In effect, this defines the “push” side of the long-running “push vs. pull” debate, which is derived in part from the desire to formulate diets for early lactation cows that minimize the extent to which milk nutrient output is derived from body tissue. Here, we will

consider progress over the last 25 yr in developing techniques and acquiring measurements of nutrient absorption and delivery to peripheral tissues in dairy cows and other ruminants, the development of mathematical models of nutrient use in dairy cows, and our understanding of the environmental by genetic interactions (push vs. pull) that determine production and nutrient partitioning in modern dairy cows.

In his review 25 yr ago, Moe highlighted the considerable progress made in using calorimetry to develop a database of energy metabolism measurements that were used for the subsequent refinement of existing feeding standards for dairy cows. However, his view at the time was that measurements of the absorption of specific products of digestion were crucial for further improvements to be made in those feeding standards. In a sense, many felt that the existing “black-box” systems of rationing energy and protein needed to be replaced with nutrient-based systems before the “Holy Grail” of predicting performance and milk composition could be reached. At the time, use of duodenal cannulation techniques to measure nutrient flow from the rumen was yielding vitally important data describing site of digestion in cattle. In the subsequent 25 yr, the database of measurements of ruminal and postruminal digestion of protein, starch, and fiber has grown tremendously, although descriptions of nutrients actually disappearing from the small intestine of lactating dairy cows were, and still are, lacking. This in part reflects the difficulty of surgically establishing and successfully maintaining the ileal cannulas required to quantify disappearance in the small intestine.

As an alternative to measuring nutrient disappearance from the lumen of the gut, the apparent absorption of nutrients into the portal vein can be quantified using multicatheterization techniques pioneered in sheep by E. N. Bergman from Cornell University in the 1960s and 1970s, and advanced in cattle in the 1970s and 1980s by D. B. Baird and colleagues at Compton in England and G. B. Huntington and colleagues at USDA in Beltsville, MD. These techniques enable the measurement of venous-arterial concentration differences and blood flow across the tissues of the portal-drained viscera (PDV; including the gastrointestinal tract, pancreas, spleen and associated adipose) or sections of the PDV, such as the section of the intestines and adipose drained by the anterior mesenteric vein (the mesenteric-drained viscera). These measurements equate to the net amount of specific nutrients, or their metabolites, absorbed into the portal vein and available to other body tissues after metabolism by the tissues included in the venous drainage (Figure 1).

All portal vein blood must traverse the liver before reaching the heart and then the rest of the body; conse-

Figure 1. Stylized view of the splanchnic vasculature; arrows show direction of blood flow.

quently, liver metabolism has an immediate impact on the availability of many nutrients absorbed into the portal vein. By placing a catheter into a hepatic vein, measurements of net liver flux can also be obtained. Together, the PDV and liver are often referred to as the “total splanchnic” tissues. In quantitative terms, the net release of an absorbed nutrient across these tissues represents the net availability of that nutrient for other body functions, such as milk synthesis. On the other hand, net removal of a nutrient across the total splanchnic bed represents the net use of nutrients derived from other body tissues, as can occur for lactate in early lactation, because it is used for glucose synthesis in the liver. In this case, a negative lactate flux indicates that liver uptake is greater than the net appearance across the PDV.

To properly interpret and integrate measurements of net nutrient flux across the splanchnic tissues with nutrient metabolism by other tissues, it is critical to be cognizant at all times that these are net measurements. For example, net flux of glucose across the PDV of dairy cows is often zero or slightly negative, but this does not mean there is no glucose absorption, as one might initially conclude. Indeed, the low recovery of starch provided postruminally to dairy cows as increased net absorption of glucose across the PDV led many to the logical conclusion that the amount of starch absorbed from the small intestine as glucose was low, or that there was substantial loss of glucose during absorption due to use by small intestinal enterocytes. However, a net flux of zero also may mean that the amount of glucose removed from arterial blood by the PDV is equal to the amount of glucose absorbed by the small intestine and released into the portal vein. Indeed, recent studies by D. L. Harmon and colleagues at the University of

Kentucky have found that increases in glucose absorption into the portal vein are balanced in part by increases in the use of glucose from arterial blood. The use of arterial glucose by the PDV accounts for as much as 25% of whole body turnover, in part because of the substantial adipose tissue included in the PDV.

To obtain measurements of true rates of nutrient absorption and release into venous blood, and removal from arterial blood, researchers can combine measurements of blood flow and venous-arterial difference with nutrient-labeling methodologies. The combination of multicatheterization and labeling techniques provides a powerful tool for measuring the complexities of metabolism of a nutrient in vivo under varying dietary and physiological conditions. Depending on the nutrient labeled, measurements of whole body and tissue fluxes can be obtained, as well as amounts of the nutrient oxidized and the interconversions with specific metabolites on a whole-body and specific-tissue basis. Historically, radioactive isotopes were used to label specific nutrients and the products of their metabolism and determine rates of unidirectional or gross metabolism across specific tissues. More recently, the development of mass spectrometry for measurements of stable isotope enrichment and the commercial availability of a variety of metabolites labeled with stable isotopes has provided methods for isotopic labeling that do not pose a health and safety risk. These events have led to the virtual replacement of radioisotopes with stable isotopes in studies of nutrient turnover and flux across splanchnic tissues.

The last 25 yr has seen remarkable progress both in the improvement and refinement of techniques for measuring nutrient absorption and metabolism by the splanchnic tissues, and in the use of the technique to

quantify the impact of dietary composition and physiological state on nutrient absorption and metabolism. In general, the technique has progressed from the stage where very short-lived catheter patency meant that many studies were conducted in a limited number of animals within days after surgery, with obvious implications for the robustness and applicability of the data obtained. With improvements in techniques, technologies, and experience of the researchers, the approach has progressed such that multicatheterization preparations in dairy cattle enable viable measurements of the effects of physiological state, dietary composition, or strategic nutrient supply to the lumen of the gut via infusions over periods of months, and in many cases for multiple lactations. Although some innovations in blood flow measurement have occurred, the improved success and viability of the approach for measurements of splanchnic metabolism most likely are not a consequence of any specific new technologies or catheter materials, but to a large extent reflect the experience of laboratories that have made a sustained commitment to application of the techniques.

The NRC system for rationing energy to dairy cows using net energy of lactation was based on a statistical integration of roughly 500 measurements of energy balance obtained using respiration calorimetry. During the last 25 yr, many more measurements of net nutrient absorption and metabolism by the splanchnic tissues of cattle and sheep have been obtained, although far fewer measurements are available for lactating dairy cows. In contrast to the Beltsville data set describing energy metabolism, which was obtained using similar experimental techniques at one location, measurements of splanchnic metabolism from individual trials and laboratories are subject to considerable experimental variance that must be accounted for in any mathematical integration of the data. Sources of variation include factors such as catheter sampling tip placement, sampling frequency, animal management, and analytical techniques. However, one overriding conclusion from the available data is the extremely intense metabolic activity of the splanchnic tissues. In studies in which both splanchnic and whole body oxygen consumption have been measured simultaneously, the PDV and liver account for 40 to 55% of body oxygen consumption, with the 2 tissue beds accounting for roughly equal portions. In addition, oxidative metabolism of splanchnic tissues accounts for a large portion of heat increment, as well as differences in the efficiency of energy use between forages and concentrates. The intense oxidative metabolism of these tissues is highlighted by the fact that the PDV and liver account for only 10 and 3%, respectively, of body mass. This high rate of metabolism reflects the numerous service func-

tions performed, including digestion and absorption, synthesis of large amounts of protein, and, in liver, glucose and urea synthesis. High rates of oxygen consumption and carbon dioxide production require high rates of blood flow; blood flow through the liver of a dairy cow in early lactation can be nearly 3,000 L/h.

The intense metabolic activity of these tissues, and their anatomical location between sites of digestion and entry of nutrients into the arterial blood pool, has led many to assume that there must be considerable metabolism of absorbed nutrients, which therefore limits their availability to other body tissues such as mammary gland. This assumption is supported by measurements of net nutrient absorption across the PDV, which typically demonstrate smaller quantities of individual nutrients (VFA, glucose, amino acids) absorbed into the portal vein than have disappeared from the lumen of the gut. However, it is important to remember that the PDV represents a heterogeneous collection of tissues, the majority of which do not have access to nutrients during their absorption, but must rely on arterial blood to provide the nutrients they require. The high rate of blood flow across splanchnic tissues means that these tissues receive 40% or more of cardiac output, and thus have access to the same proportion of the nutrient pool in arterial blood. When the extraction of nutrients from arterial blood is accounted for, rates of absorption of VFA, glucose, and essential amino acids from the lumen account for a much greater portion of net absorption, which indicates considerably less metabolism during nutrient absorption than previously assumed. Although a substantial use of some nonessential amino acids and VFA occurs during their absorption, their nitrogen and carbon are in part repackaged as alanine and ketone bodies, which are made available to other tissues. Although in liver there may be a substantial extraction of individual amino acids from blood, this largely represents use of amino acids derived from arterial blood, rather than metabolism of amino acids during their first pass through the liver after absorption into the portal vein. Absorbed propionate and *n*-butyrate are extensively removed by the liver, but are largely repackaged as glucose and β -hydroxybutyrate, respectively, which are released to the periphery.

The observation that splanchnic tissues derive the majority of their nutrient requirements from the arterial pool has important implications for mathematical models of nutrient metabolism. Rather than restricting entry of nutrients, it now appears that the splanchnic tissues largely compete with other body tissues for nutrients from the same arterial blood pool. Consequently, metabolism of nutrients in PDV and liver will be subject to the same regulatory controls as for other tissues, and is in part determined by the supply of nutrients from the

diet relative to demand. Therefore, for future models of nutrient use to predict the use of absorbed nutrients, the propensity of the mammary gland and other tissues for productive nutrient use, or the "pull" effect, must be represented mathematically. Numerous studies of the use of amino acids supplied via postruminal or intravenous infusion have found that unless amino acids are limiting production, the provision of supplemental essential amino acids has a greater effect on liver urea production and urinary nitrogen excretion than on milk protein output. This should not be surprising to anyone with a rudimentary knowledge of amino acid nutrition in nonruminants. Similarly, increased supply of glucose and other energy substrates does not necessarily "push" more milk component yield, but may increase body energy deposition instead. The response will very much depend on the productive and physiological state of the cow, as demonstrated, for example, by the effects of exogenous bovine somatotropin administration.

Over the last 25 yr, measurements of splanchnic metabolism have illuminated the "black box" of nutrient absorption and metabolism within the cow, and an increasingly robust database of nutrient flux across the PDV and liver with which to parameterize mechanistic models of nutrient use has been generated. These measurements are crucial to our understanding of the effects of diet composition on nutrient absorption and liver metabolism. For example, patterns of VFA absorption into blood can only be assessed using multicatheterization techniques, as the liver removes most of the VFA absorbed during first-pass metabolism. Similarly, patterns of gut and pancreatic hormone release into the portal vein are not necessarily reflected by changes in their peripheral concentrations due to extensive liver removal from portal vein blood. When mathematical models fail to predict patterns of nutrient metabolism observed *in vivo*, e.g., net acetate release by the liver, then reasons for that failure can be addressed by including additional levels of control at appropriate points in enzymatic pathways.

Multicatheterization techniques have also been used to address hypotheses relevant to specific aspects of digestion and metabolism in ruminants. A nonexhaustive list of examples include the considerable body of work addressing the capacity for postruminal starch digestion and glucose absorption, the extent of VFA metabolism by ruminal tissues, the effect of increased ammonia absorption on liver metabolism, rates of nitrogen cycling between the PDV and liver, and studies of factors influencing amino acid absorption and metabolism by the PDV and liver of lactating dairy cows. Although many important research hypotheses have been addressed by these studies, in many cases their greatest contribution is the enhanced understanding they pro-

vide of basic metabolic processes, which may have more long-term benefit than the answers they provided to the questions that originally justified the research.

PROGRESS IN UNDERSTANDING TISSUE METABOLISM

In parallel with the major advancements provided by multicatheterization studies, the past quarter-century has seen progress in characterizing and quantifying nutrient metabolism by the tissues of key organs, including the mammary gland, adipose tissue, and liver. Two major outcomes of this research have been realized that complement nutrient flux data. One is the quantitative integration of endogenous nutrients provided by mobilization of lipids from adipose tissue and protein from skeletal muscle and reproductive tract with dietary nutrients provided by the digestive tract. The second is the quantitative modeling of responses of the mammary gland and other tissues to differences in nutrient supply brought about by metabolic events in support tissues.

In vitro tissue approaches can provide useful estimates of *in vivo* enzymatic activities and responses to control mechanisms. For example, R. L. Baldwin and colleagues at the University of California at Davis used *in vitro* techniques to better establish fundamental nutrient use by the mammary gland. As one example, they used slices of mammary tissue incubated *in vitro* with various concentrations and combinations of key substrate molecules such as glucose, acetate, and lactate. By using a range of concentrations of substrates that were labeled with radioactive tracers in different molecular positions, they were able to provide quantitative estimates of kinetic constants for metabolism of substrates through various important pathways such as the citric acid cycle, the pentose phosphate pathway, and *de novo* lipogenesis. These kinetic data were then used to expand their mathematical models of mammary metabolism. This group also used arteriovenous concentration differences in blood across the mammary gland to provide estimates of uptake and metabolism of amino acids and energy-yielding substrates by the mammary gland.

Metabolic activities of tissues that support milk production can also be determined using *in vitro* techniques. During the 1980s, John McNamara and colleagues at Washington State University established an extensive quantitative description of adipose tissue metabolism over the entire lactation cycle, and how functions of lipid synthesis and mobilization are affected by stage of lactation, parity, and diet composition. Their approach was to measure responses of subcutaneous adipose tissue obtained by repeated biopsy across de-

defined timepoints in lactation from cows of different genetic backgrounds and fed at different energetic intensities. Use of tissue slices incubated with radiolabeled substrates showed, for example, that fatty acid synthesis from acetate at 60 d of lactation was lower for cows of high genetic merit for milk production than for those of lower genetic merit. Lipolytic responses generally were higher throughout lactation than during the dry period and were enhanced in high genetic merit animals. Together, the corresponding changes in lipid synthesis and lipid mobilization in adipose point to the predisposition for high genetic merit cows to divert more energy toward milk synthesis than their lower-yielding contemporaries. The Washington State University scientists also integrated tissue-level metabolism with key enzymatic and endocrine control mechanisms. Although not the direct focus of this article, a tremendous increase in knowledge of physiology and the role of the endocrine system in partitioning use of energy-yielding nutrients and amino acids has been central to enhanced understanding of fundamental nutrition; advances in endocrine physiology are discussed elsewhere in this issue.

The previous 25 yr has seen an explosion of knowledge on metabolism in the liver of dairy cows, a substantial portion of which has been generated using *in vitro* approaches. Knowledge of changes in direction and magnitude of various pathways of fatty acid, glucose, and amino acid metabolism has progressed substantially, driven largely by the need to understand adaptations of the liver during the transition from pregnancy to lactation as discussed earlier. During the periparturient period, the liver becomes flooded with NEFA from adipose tissue that must be oxidized, converted to ketone bodies, or reesterified to TG (Figure 2). Research over the last 25 yr has clearly shown that the low rate of synthesis and secretion of very low density lipoproteins (VLDL) by ruminants favor accumulation of TG in the cell when TG is being actively synthesized. Groups at the University of Wisconsin, Iowa State University, University of Illinois, Michigan State University, Cornell University, and Purdue University among others have used various *in vitro* systems, including isolated hepatocytes, liver slices, or tissue homogenates to quantify metabolic pathways as affected by substrate and metabolite supplies, hormonal influences, and physiological status of the donor animal. Findings from this collection of research have contributed to enhanced understanding of the integration of carbohydrate, fatty acid, and amino acid metabolism in healthy and diseased states. Baldwin's group from the University of California at Davis used similar approaches to that described for mammary slices to obtain estimates of key kinetic parameters needed to model hepatic metab-

olism. Together with whole-animal, splanchnic metabolism, and molecular data, tissue metabolism data obtained during the last 25 yr have greatly enhanced our understanding of fundamental nutrition in dairy cattle. In the future, *in vitro* approaches can be used to address some of the questions raised by the multicatheterization studies, and vice versa.

PROGRESS IN MOLECULAR REGULATION OF METABOLISM

Background and Context

During the past 25 yr there has been an important shift in focus of nutrition research to include molecular biology and genetics. The shift began when Paul Berg of Stanford University generated the first modified DNA molecules in 1972. This pioneering work, for which Berg was awarded the 1980 Nobel Prize in chemistry, combined the DNA of 2 organisms (human and *Escherichia coli* bacteria) and created the first recombinant DNA molecule. The ability to find the minimal sequence of DNA that codes for a protein was made possible by the discovery of reverse transcriptase, a retroviral enzyme that copies RNA to DNA, by Howard Temin and David Baltimore in 1970. Consequently, when mRNA is isolated from cells and reverse-transcribed, it generates a DNA strand that is complementary (cDNA) to the mRNA made by that cell (or tissue). These milestone achievements and many others resulted in the emergence of recombinant DNA technology and provided the primary tools for analysis of mammalian gene structure, function, and subsequent profiling tools now used to understand the interplay between nutrition and the mammalian genome.

The central dogma of molecular biology states that DNA makes RNA makes protein. The application of molecular biology to understanding nutrient metabolism centers on identifying conditions that lead to the amplification of genetic information and synthesis of several copies of RNA and ultimately the initiation and progression of protein synthesis. The ultimate end point of these processes in dairy cows is a coordinated change in cellular and tissue metabolism to maintain homeostasis or to undergo homeorhesis to support a new physiological state.

With few exceptions, every cell of the body contains a full set of chromosomes and identical genes yet only a portion of these genes is expressed in each cell type. The genes that are expressed confer the unique properties to each cell type. The regulation of gene expression therefore can be affected by specific controls at each step between DNA transcription and protein synthesis. The more elements there are in the pathway, the more opportunities there are for control with different cir-

Figure 2. Schematic of metabolic relationships among adipose tissue, liver, and mammary gland during the transition period; NE = norepinephrine, Epi = epinephrine, CPT-1 = carnitine palmitoyltransferase-1, GPAT = glycerol-3-phosphate acyltransferase, TG = triglyceride, CoA = coenzyme A, VLDL = very low density lipoprotein.

cumstances. For example, control of expression of a gene that encodes a key enzyme in metabolism may be exerted by binding of a nuclear protein or transcription factors to promoter sequences within the 5' region of the DNA that contains the genetic information for the enzyme. A classic example of this form of control is activation of phosphoenolpyruvate carboxykinase (**PEPCK**) gene expression by glucagon, which involves binding of the cyclic-AMP responsive element binding protein to the PEPCK promoter. Occupation of these DNA elements by a transcription factor then leads to an increase in the rate of transcription of the gene through recruitment of other proteins and RNA polymerase. Conversely, a nuclear protein can also bind DNA and act to repress expression of gene transcription by blocking transcription initiation.

A second level of control exists in the structure of the mRNA transcripts synthesized from the DNA template. These mRNA contain the protein coding information as well as flanking nucleotide sequences. The 5' and 3' untranslated sequences as well as the three-dimensional structure of the mRNA play an important role in maintaining mRNA stability and the rate of association of the mRNA with the cell's protein synthetic machinery. For example, 5' regions of pyruvate carboxyl-

ase (**PC**) mRNA contain elements that affect the association of PC mRNA with the ribosomal subunits and therefore the rate of translation of the PC enzyme. Proteins that have been identified as regulatory enzymes for key metabolic pathways, and the regulation of enzymatic activity, are a third level of control. Considerable study in this area has helped to identify flux-generating steps for several metabolic processes in ruminants. Application of molecular biology tools serves to complement rather than supersede earlier approaches. Furthermore, it is essential to appreciate that nutrient metabolism is potentially controlled at all 3 levels and that all levels of control can respond to changes in nutrient supply.

Application of molecular tools to understanding nutrient metabolism has primarily used mRNA transcript analysis and linked the changes in mRNA to metabolic activity, physiological state, or nutritional status. In most instances, the candidate transcripts are selected based on knowledge of the central importance of the encoded protein in controlling a particular metabolic process. Transcript analysis under these conditions provides additional information on the origins of control of the pathway. More recent use of whole-animal and tissue-specific gene knock-out and over-expression

models have provided additional insight to the impact of key metabolic reactions on whole animal and tissue metabolism, but application of these technologies to date has mainly been limited to rodent models.

The majority of research on nutrient regulation of gene expression involves use of DNA or RNA probes or amplification assays, such as PCR and real-time PCR. Recently developed array-based screening tools represent an evolution of these basic approaches. Hybridization, or the process of joining 2 complementary strands of DNA or RNA through base pairing is the core process necessary for detection methods to quantify abundance of a particular transcript. In a classical Northern blot analysis of mRNA, a single-stranded DNA or RNA molecule with a known nucleotide sequence is used to detect a complementary base sequence that has been immobilized to a solid support. When bound to its target, the probe is then detected with a radioactive, fluorescent, enzymatic, or chemiluminescent molecule so it can be visualized. A variation of this principle includes ribonuclease protection assays in which the probe and target complex is formed in a solution and then separated for quantification of the mRNA transcript.

An important point to note is that analysis of candidate genes relies on the nucleotide sequence information for the transcript of interest or the availability of a closely related cDNA from another species. The sequence and assembly of genomes of various organisms has hastened the identification of genes that are expressed in response to physiological state, nutritional status, and hormonal changes.

Transcript profiling using DNA microarrays or candidate gene analysis is a static measure of the pool size of individual mRNA in a cell or tissue and does not provide any information on the dynamics of mRNA synthesis (transcription) or RNA degradation (RNA stability). Determining the rate of mRNA synthesis typically involves use of nuclear run-on assays that measure the relative amount of new transcripts made from the previously initiated RNA polymerases and provides a measure of the *in vivo* rate of transcription of DNA to mRNA. Posttranscriptional modification of RNA through the addition of a 5' cap and a polyadenylation (polyA) tail protect RNA from exonucleases and aids in recognition of the mRNA by the translational machinery of the cell. The rate of degradation of mRNA is initiated by a gradual shortening of the polyA tail and removal of the 5' cap structure. The half-life of RNA is determined by *in situ* hybridization histochemistry, Northern blot analysis, or quantitative real-time PCR analysis of RNA samples removed from cells treated with transcription inhibitors such as actinomycin D or α -amanitin. Levels of some key proteins (e.g., the transferrin receptor) are regulated at the level of RNA stabil-

ity; however, measures of RNA degradation rate are presently not easily attainable *in vivo* and therefore nutritional impacts on gene expression usually are only described for transcription rate and mRNA abundance.

Areas of Investigation and Major Milestones in Dairy Cattle Nutrition Research

Although the application of molecular research tools to improving our understanding of the regulation of nutrient metabolism in dairy cattle has grown over the past 25 yr it can still be considered in a state of infancy. Some of the discoveries during the past quarter-century that have used molecular biology techniques to better understand the nutritional physiology of dairy cattle are described below as examples. In many cases, the impetus for these investigations has been the knowledge of flux control and identification of key metabolic reactions identified during the first 75 yr of research in dairy science. Increased application of molecular research tools toward Moe's goal of "understanding of how animals respond to variations in amounts of key nutrients absorbed from the gut" is inevitable and will lead to the development of molecular reagents and protocols that are specific to the bovine.

The availability of glucose or glucose precursors has a profound effect on milk production and animal health. Control of glucose transporter expression in the gut and other tissues has been investigated in dairy cattle in an attempt to understand limitations in glucose absorption when diets contain a high portion of cereal grains. Researchers from John Kennelly's group at the University of Alberta found that sodium-dependent glucose transporter 1 (SGLT1) mRNA is found along the gastrointestinal tract including the rumen, omasum, duodenum, jejunum, ileum, and cecum of dairy cows. Infusion studies revealed that the presence of glucose in the intestine increases SGLT1 mRNA abundance by 2-fold, compared with a 60- to 90-fold increase in cotransporter number and activity. These data highlight the importance of translational and posttranslational modification as primary modes of regulation of glucose absorptive capacity.

Facilitated glucose transporters are proteins that function in the postabsorptive exchange of glucose between blood and tissues. The glucose transporter (GLUT) isoforms are membrane proteins derived from a family of closely related genes that differ in their tissue expression. The GLUT-1 protein is considered a ubiquitously expressed glucose transporter and GLUT-1 mRNA has been found in all bovine tissues examined except liver. Expression of GLUT-1, -2, -3, -4, and -5 in bovine follow the tissue-specific expression patterns that have also been observed in humans, whereas ex-

pression of mRNA for GLUT-3 and GLUT-5 may be unique to bovine mammary gland.

Insulin-dependent glucose transport is mediated through changes in compartmentalization of GLUT-4 within cells and GLUT-4 gene expression. The fact that GLUT-4 is not expressed in bovine mammary epithelial cells supports a lack of insulin-responsive glucose uptake by mammary tissue as confirmed using other approaches. A portion of the effect of somatotropin to increase milk yield involves a repartitioning of glucose away from adipose tissue and muscle and toward mammary gland through changes in muscle GLUT-4 expression. Although GLUT-3 and GLUT-5 have been identified in bovine liver, the expression of GLUT-7, the primary transporter of glucose from the hepatocyte, has not been confirmed. In nonruminants, GLUT-7 serves to transport glucose-6-phosphate to the endoplasmic reticulum where glucose-6-phosphatase acts to release free glucose into the cytoplasm. Bovine liver may offer unique opportunities to study the regulation of GLUT-7 when one considers the importance of hepatic gluconeogenesis in lactating dairy cows and that glucokinase activity, the primary opposing reaction to cellular glucose release, is essentially absent.

A fundamental response to nutrient supply is a change in signals that regulate metabolic pathways such as hormones or the direct and indirect actions of key nutrients and metabolites to alter metabolism through changes in gene expression. Nutrient deprivation is one of the most effective experimental tools in identifying genes that respond to nutritional status, due perhaps to the close association between nutrient supply and hormonal status. Likewise, feed restriction protocols have been used to study the feedback mechanisms that control feed intake. The decrease during short-term feed deprivation in circulating concentrations of cholecystokinin and glucagon-like peptide 1, gastrointestinal hormones that may play a role in feeding behavior, is due to a reduction in their mRNA abundance at the duodenum and ileum. Yves Boisclair's research group and others have shown that leptin, a hormone secreted primarily by adipose tissue, plays a role in energy homeostasis and several other physiological functions in dairy cattle. Leptin mRNA is expressed in several fat depots as well as mammary parenchyma. Circulating leptin concentrations are reduced in cattle after 24 h of feed deprivation and in cattle during the transition to lactation. The decrease in leptin concentration is associated with a decrease in leptin mRNA in adipose tissue. The response to leptin depends on the tissue distribution and level of expression of its receptor in target tissues. The long-form and short-form of the leptin receptors are expressed in a tissue specific manner in dairy cattle and expression patterns of the

2 forms likely determine the physiological actions of leptin in those tissues, especially when coupled with the complement of hormones and growth factors that modulate its activity.

As discussed earlier, considerable progress has been achieved in understanding the physiology associated with the transition to lactation and a model of the underlying molecular mechanisms that accompany this homeorhetic adaptation is beginning to emerge. The recognition that fatty liver is prevalent in dairy cows and can impair lactation performance coupled with the recognition of a reduced rate of export of TG from ruminant liver has prompted research on molecular processes associated with secretion of lipids as VLDL. A decrease in expression of apolipoprotein B₁₀₀ in periparturient dairy cows is consistent with a reduction in VLDL synthesis and suggests that liver lipid infiltration may be partially regulated at a transcriptional level. Other studies have eliminated steps of the VLDL assembly process such as microsomal transfer protein mRNA expression as contributors to this pathology.

Cloning and characterization of PEPCK, a key enzyme for gluconeogenesis in dairy cattle, reveals a close association between the cytosolic form of PEPCK and activity of the enzyme in bovine liver, similar to nonruminants. Expression of PEPCK is increased as lactation progresses toward peak milk and is increased in response to somatotropin at the level of transcription of the gene. Similarly, the cloning and characterization of PC, a key enzyme in oxaloacetate regeneration in the cell and in gluconeogenesis from lactate and alanine, indicates a link between enzyme activity and mRNA expression in liver from transition cows. Increased PC mRNA abundance at calving supports an increased flux of lactate and alanine carbon to glucose measured using transorgan balance techniques as well as increased flux of alanine to glucose measured in liver slices (Figure 3). The presence of six 5' transcript variants for bovine PC is consistent with an additional level of regulation of PC activity through changes in the translational efficiency of mRNA as also observed in nonruminants.

The recognition that fatty acids are regulators of metabolism and act in the cell nucleus to control expression of genes for fatty acid synthesis has been extended to investigations on the origins of milk fat depression in dairy cows. For example, Kennelly's group at the University of Alberta found that the reduction in milk fat when fish oil is fed is linked to decreased mRNA abundance of acetyl CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase (also known as Δ^9 -desaturase). Bauman's group at Cornell showed that the potent action of *trans*-10, *cis*-12 CLA to reduce milk fat synthesis in lactating dairy cows involves a reduction in mRNA for acetyl CoA carboxylase, fatty acid synthase,

Figure 3. Example of how data from different studies using different approaches can provide complementary information to the same metabolic issue. These data demonstrate that contribution of alanine and lactate to glucose synthesis is greater around calving in dairy cows, and that this contribution likely is regulated by the key enzyme pyruvate carboxylase. The top panel shows the maximum amount of glucose made by the liver that could be synthesized from lactate or alanine as measured by splanchnic nutrient flux techniques in multicatheterized cows (data from Reynolds et al., 2003). The middle panel shows the rates of conversion of radiolabeled alanine (expressed as micromoles per hour per gram of wet liver) to glucose in liver slices obtained by biopsy (data from Overton et al., 1998). The bottom panel shows relative abundance of mRNA for pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) measured in liver obtained by biopsy (redrawn from Greenfield et al., 2000). Relative conversion of lactate and alanine, both of which are converted to pyruvate for subsequent conversion to glucose, is greater around and shortly after calving, which corresponds to greater expression of PC.

stearoyl-CoA desaturase, lipoprotein lipase, fatty acid binding protein, and glycerol phosphate acyltransferase. These changes are directly linked to a substantial reduction in lipogenic activity in explant cultures *in vitro*. These data provide understanding of the molecular basis for milk fat depression, a practical milk production problem that has been recognized for several decades.

The majority of advances in our understanding of nutrient regulation of gene expression have focused on candidate gene analysis. Microarray technology has enabled investigation of genome-wide transcript profiles to determine the expression patterns of thousands of genes simultaneously. Early reports of application of microarray technology for identifying differential gene expression patterns with physiological state and nutritional status appear promising and considerable effort is underway currently by several research groups. Refinements in sample handling, statistical analysis, and hierarchical clustering of expressed transcripts and development of bovine-specific metabolic maps will undoubtedly lead to a greater understanding of the complex molecular basis of nutrient metabolism in support of milk production.

FUTURE PERSPECTIVES

Considerable progress has been made toward the goals of better understanding and prediction of absorbed nutrient use envisioned by Moe and others 25 yr ago. However, much remains to be learned, and it seems doubtful that available technology and mathematical representations alone will achieve the "Holy Grail" of predicting the subtleties of productive responses (e.g., milk protein or fat yield) to changes in diet composition, environmental influences, and physiological state. Indeed, the marriage of measurements of the "omics" technologies (genomic, proteomic and metabolomic responses) with measurements of tissue metabolism will be enlightening, and the identification of key responses to changes in diet, management, physiological state, and genetics may well lead to technologies for predictive screening of the propensity for productive nutrient use in individual cows in a practical setting.

A shift in focus of nutrition research toward molecular biology and genetics coupled with development of tools to study the response of the genome to nutrition has led to a blended discipline known as nutritional genomics or "nutrigenomics." Application of this approach to dairy cattle nutrition is currently in its infancy and will build on the candidate gene analysis that forms the bulk of our knowledge of molecular regulation of nutrient metabolism. There are high expectations that nutrigenomics, when coupled successfully with

more traditional methodologies, will provide previously unreachable information on the dynamics of metabolic control in response to nutrient supply, will serve to clarify the causal factors of metabolic disorders, and will shape future feeding strategies to enhance productive efficiency and animal well-being during the next 25 yr.

REFERENCES

- Agca, C., C. A. Bidwell, and S. S. Donkin. 2004. Cloning of bovine pyruvate carboxylase and 5' untranslated region variants. *Anim. Biotechnol.* 15:47–66.
- Agca, C., R. B. Greenfield, J. R. Hartwell, and S. S. Donkin. 2002. Cloning and characterization of bovine cytosolic and mitochondrial PEPCK during transition to lactation. *Physiol. Genomics* 11:53–63.
- Ahnadi, C. E., N. Beswick, L. Delbecchi, J. J. Kennelly, and P. Lacasse. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. *J. Dairy Res.* 69:521–531.
- Baird, G. D., H. W. Symonds, and R. Ash. 1975. Some observations on metabolite production and utilization in vivo by the gut and liver of adult dairy cows. *J. Agric. Sci.* 85:281–296.
- Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. *Trans*-10, *cis*-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *J. Dairy Sci.* 85:2155–2163.
- Bell, A. W., R. Slepatis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. *J. Dairy Sci.* 78:1954–1961.
- Bergman, E. N. 1975. Production and utilization of metabolites by the alimentary tract. Pages 292–305 in *Digestion and Metabolism in the Ruminant*. I. W. McDonald and A. C. I. Warner, ed. New England Publishing Unit, Armidale, Australia.
- Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. *J. Dairy Sci.* 75:1914–1922.
- Blome, R. M., J. K. Drackley, F. K. McKeith, M. F. Hutjens, and G. C. McCoy. 2003. Growth, nutrient utilization, and body composition of dairy calves fed milk replacers containing different amounts of protein. *J. Anim. Sci.* 81:1641–1655.
- Blouin, J. P., J. F. Bernier, C. K. Reynolds, G. E. Lobley, P. Dubreuil, and H. Lapierre. 2002. Effect of diet quality on splanchnic fluxes of nutrients and hormones in lactating dairy cows. *J. Dairy Sci.* 85:2618–2630.
- 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.
- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259–2273.
- Forsberg, N. E., R. L. Baldwin, and N. E. Smith. 1984. Roles of acetate and its interactions with glucose and lactate in cow mammary tissue. *J. Dairy Sci.* 67:2247–2254.
- Greenfield, R. B., M. J. Cecava, and S. S. Donkin. 2000. Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. *J. Dairy Sci.* 83:1228–1236.
- Hanigan, M. D., L. A. Crompton, C. K. Reynolds, D. Wray-Cahen, M. A. Lomax, and J. France. 2004. An integrative model of amino acid metabolism in the liver of the lactating dairy cow. *J. Theor. Biol.* 228:271–289.
- Harmon, D. L., C. J. Richards, K. C. Swanson, J. A. Howell, J. C. Matthews, A. D. True, G. B. Huntington, S. A. Gahr, and R. W. Russell. 2001. Influence of ruminal or postprandial starch on visceral glucose metabolism in steers. In: A. Chwalibog and K. Jakobsen (eds.) *Energy Metabolism in Farm Animals*. EAAP Publ. 103. p 273. Wageningen Pers, Wageningen, The Netherlands.
- Huntington, G. B. 1982. Portal blood flow and net absorption of ammonia-nitrogen, urea-nitrogen, and glucose in nonlactating Holstein cows. *J. Dairy Sci.* 65:1155–1162.
- Kristensen, N. B., and D. L. Harmon. 2004. Splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. *J. Anim. Sci.* 82:2033–2042.
- Lapierre, H., and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* 84(E Suppl.):E223–E236.
- McNamara, J. P. 1991. Regulation of adipose tissue metabolism in support of lactation. *J. Dairy Sci.* 74:706–719.
- Milano, G. D., A. Hotston-Moore, and G. E. Lobley. 2000. Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver. *Br. J. Nutr.* 83:307–315.
- Moe, P. W. 1981. Energy metabolism of dairy cattle. *J. Dairy Sci.* 64:1120–1139.
- National Research Council. 1989. *Nutrient Requirements of Dairy Cattle*. 6th rev. ed. Natl. Acad. Press, Washington, DC.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Overton, T. R., J. K. Drackley, G. N. Douglas, L. S. Emmert, and J. H. Clark. 1998. Hepatic gluconeogenesis and whole-body protein metabolism of periparturient dairy cows as affected by source of energy and intake of the prepartum diet. *J. Dairy Sci.* 81(Suppl. 1):295. (Abstr.)
- Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.* 86:1201–1217.
- Reynolds, C. K., G. B. Huntington, H. F. Tyrrell, and P. J. Reynolds. 1988. Net portal-drained visceral and hepatic metabolism of glucose, L-lactate, and nitrogenous compounds in lactating Holstein cows. *J. Dairy Sci.* 71:1803–1812.