

**RELEVANT USE OF METABOLIC MODELS TO STUDY EFFICIENCY IN DAIRY CATTLE AND APPLY IMPROVED PRACTICES ON FARMS--OR-- WHY WE REALLY NEED TO MOVE TO A FULLY SYSTEMS BIOLOGY APPROACH TO REMAIN VIABLE AND PROVIDE QUALITY HUMAN FOOD.**

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## **Introduction**

The dairy cow is an awesome animal that can produce her weight in human food every two weeks, turning low quality biomass into quality human food. The progress we have made in dairy science and in practical dairy cow management is impressive indeed. During lactation, demand for all nutrients doubles within a few days and within a week or two can be 3 to 5 times as high as in mid gestation. The primary metabolic problems are a severe shortage of glucose, amino acids, and major minerals such as calcium, phosphorous, sodium, chloride and potassium. The primary practical problems involve managing a very rapid increase in intake of a ration properly supplied and balanced with all nutrients. Within this goal is the troublesome problem of balancing the physical form of the diet to ensure sufficient intake, to discourage excess intake of energy and to supply the rumen ecosystem with inputs in the proper physico-chemical form to ensure the proper amount and balance of microorganisms. In addition, the cow needs to avoid metabolic and reproductive diseases and ideally conceive and calve again within 12-15 months and start all over. No Problem! It is amazing how well cows and dairy producers, consultants and veterinarians have responded.

But you might guess, I think we can get even better. How about a 10 year old cow that had her first calf at 36 months of age, has had 7 calves, and in her 7<sup>th</sup> lactation peaked at 220 lbs of milk and gave over 55,000 lbs of milk as a 10 year old? This cow lives in a herd of about 550 Holsteins in NSW, Australia, a farm continuously in business since 1828. Production is at 11,000 kg/305 d on a grass based TMR. The owner is disappointed her estrus detection rate is 'only' 93 %, and pregnancy rate is 'only' 53 %. So why can't we do that?

Actually, dairy nutritionists, veterinarians and managers have done an outstanding job in preparing cows for the demands of lactation. Our continued worldwide increase in dairy cattle efficiency with a large reduction in peri-parturient diseases is proof of that. With what we already understand we can do a good job of ensuring the health and welfare of the animal while allowing large quantities of milk production. *Further improvement will happen as we understand in more detail the metabolic regulation in the animal during this time and the complex interactions*

*between the physical and chemical form of the ration and the rumen microbial ecosystem.* The challenge of proper nutrition must be met by an understanding of the chemistry and utilization of a variety of plant proteins, cellulose, hemicellulose, lignin, other complex polysaccharides, starch and organic acids. Most importantly, continued improvement in nutrition will happen as we recognize that nutrition of any one compound is intrinsically linked with nutrition and metabolism of all compounds (why do you think we started feeding totally mixed rations?).

Basic management practices will change perhaps only slightly from the principles and concepts we now practice. Producers will continue to demand good ration formulation, mixing and delivery of a fresh totally mixed ration, with attention to levels of quantity and quality of protein, neutral detergent fiber, acid detergent fiber, non-fiber and non-starch polysaccharides, starch, physical form [fiber chop length, water content, starch and protein solubility, mixing], adequate bunk space, adequate fresh water, and all the rest of the nutritional, cow-comfort and disease-preventative measures we now use. Improvements will likely come through more subtle shifts and refinement of feedstuff processing [flaking, rolling, hydration, enzymatic pre-digestion, mixing] to better manage the rumen environment, and continued refinement in the balance between chemical form of the ration, the cow's voluntary feed intake, and the mechanisms of metabolic regulation that control lactation and feed intake.

Large increases in efficiency are still possible at the rumen level [fiber digestibility still is in the 50 to 60 % range, can it or should it be improved? What would be the implications for rumen health and microbial protein synthesis if we 'went too far'?]. However, we must always remember that 'carbohydrate nutrition' or 'energy nutrition' really means an adequate supply of glucose to the brain and central nervous system. In early lactation, as the mammary gland demands 5, 10, 20 times more glucose than the brain uses in a day, there are definite, coordinated, homeostatic and homeorhetic endocrine and neural systems that are activated, de-activated, attenuated and/or enhanced to ensure that glucose supply to neural tissues remains adequate. We see the effects of these signals in rates of voluntary feed intake, in rates of lipogenesis and lipolysis in the adipose tissues, in proteolysis, protein synthesis and amino acid interconversions in muscle and liver, and in the increase in supply of glucose to the mammary gland.

We will improve our practical management only as well as we understand the metabolic, hormonal and neural regulation that controls the use and interconversions of glucose, acetate, propionate, butyrate, amino acids and fatty acids. The difference between the past and now, however is that we must consider all aspects of the cow simultaneously and in an integrated fashion. Previously this was relatively impossible, but with very simple tools we can manage all the complexities of the rumen and organs simultaneously. We are required to do this in one sense as our body of knowledge and our needs of the industry demand it. Anything less is not sufficient. We have to stop thinking: protein, energy, rumen, mammary, reproduction, nutrition, genetics, etc and start to think of 'the cow'!

We must remember our primary goal is to provide a quality, nutritious milk while ensuring the health and welfare of the cow, minimizing our use of natural resources and providing a reasonable profit to the producer. In order to meet this goal, we cannot ignore, in fact we need to focus more strongly on, the endocrine and neural regulation of gluconeogenesis, lipolysis and lipogenesis, amino acid interconversions and of feed intake. An excellent way to do this is in the continued development, testing, evaluation, and challenging with real data, of dynamic, mechanistic, metabolic models of metabolism in dairy cows.

### **Brief Description of the Molly Cow model.**

Models have been in use for several decades to help engineers, physicians and scientists store massive amounts of information and describe structures, systems and processes, which, without the storage and calculation power of computers, is practically impossible. One could argue that nutrition as a science cannot progress on many fronts without computing capabilities and that practical applications on the farm would be severely limited without the use of computers. Computers, numbers and math will not replace experience and ‘common sense’; they will provide an organized history of experience to producers with which to improve their lot and that of others.

A model is a representation of reality, made to help us describe and understand the system in an orderly fashion. First, it should have a clear objective, such as: “The model prepared in this publication was designed to provide practical, situation-specific information in a user-friendly format” (NRC, 2001) or, “Develop a dynamic, mechanistic model of digestion and metabolism in lactating dairy cows suitable for evaluation of hypotheses regarding underlying energetic relationships and patterns of nutrient use” (Baldwin, 1995). The U. S. National Research Council “Requirements of Animals” series have been using mathematical models since the 1940’s, improving and expanding them as data and knowledge increases. The Cornell Net Carbohydrate and Protein System, now developed into the Cornell-Penn-Miner Program for dairy management and nutrition came from the original Molly but with a purpose of more direct on farm use(CPM-Dairy; (Boston et al., 2000)).

Another key characteristic of a model is how it describes change over time. A model that describes a process at one time is static. This is true even if the ‘time’ was a growth or lactation phase extending several months. That does not make the model ‘dynamic’, it just provides a static picture of a certain period of time. A dynamic model *integrates* change over time. Both are very useful, however, I argue strongly that only a dynamic model will help us truly improve our nutritional understanding. This is primarily because the requirements for any one time or short time period are always partially a function of what has come before.

For example, the requirements in early lactation are a function in part of the situation the animal was in 60 or 30 or 10 days prior to lactation. The same can be noted

for any time period in lactation-the state of the animal is a function of the previous conditions, and the requirements for any animal are a function of its state. A cow producing 50 kg of milk at 200 days of lactation with a body condition score of 1.5 , a rapid metabolic rate in visceral tissues and a very rapid turnover rate of body fat and protein has a different requirement *for the total body* than a cow producing 50 kg of the same quality milk but with a body condition score of 3 (about 65 kg of body fat), a somewhat lower rate of visceral metabolism and a lower turnover rate of body protein and fat, although the requirement for the milk output may be the same. Static models can incorporate some of these effects of time by adding more equations relating to previous condition, but they are still static-they cannot describe the process over time.

### **Integration of nutrition, ruminal ecosystem and metabolism in a dairy cow model**

The model of Baldwin inputs chemical components of the diet: soluble carbohydrate (Sc), organic acids (Oa), pectin (Pe), lactic acid (La), lipid (Li), starch (St), hemicellulose (Hc), cellulose (Ce), soluble protein (Ps), insoluble protein (Pi), non-protein nitrogen (Nn), lignin (Lg), soluble ash (As), insoluble ash (Ai) and added fat (Ft). It also has provisions for feed acetate and butyrate for high silage diets, and urea. It also includes factors for the starch solubility, particle size and to calculate organic matter. For example from a corn/soybean:alfalfa ration (50 % forage) comes:

fDSc=0.06,fDOa=0.05,fDPe=0.06,fDLa=0.0,fDLi=0.04,fDSt=0.25,fDHc=0.09,fDCe=0.18,fDPs=0.04,fDPi=0.08, fDNn=0.03,fDLg=0.04,fDAs=0.04, fDAi=0.04, fDAc=0.0, fDBu=0.0, fdUr=0.0, stsol=0.2, PSF=0.4, fdfat=0.0, fDOM=1.0-fDAi-fDAs

The model describes most of the practical feeding strategies and intake estimates, based on either single or multiple meals per day, a specified feeding rate (usually used for simulating research trials where intake is measured), feed based on 1 kg of feed intake for each 3 kg of milk, two different equations used by earlier NRC versions based on actual data from thousands of records (Ely or Mertens equations), and several others. The point is if you have feed intake data you can simulate it. If you want to use basic accepted equations to describe intake, you can do that.

### **Description of Ruminal Processes**

Once feed is delivered to the rumen, the model partitions it as you would expect: soluble carbohydrate, cellulose, hemicellulose, organic acids (we will stick just to carbohydrates for this example). Starch can be set at various solubilities, that which is poorly soluble will pass from the rumen and that which is solubilized will be supplied totally for microbial fermentation. Cellulose and hemicellulose are partitioned to large and small particles based on the physical characteristics of the ration plus rumination (see Baldwin, 1987a, b, c or Baldwin 1995 for details).

Soluble carbohydrate (hexose equivalents) is the sum of entry of soluble sugars, starch breakdown, other carbohydrate breakdown, release from hemicellulose, release

from cellulose, fermentation by microbes, incorporation into microbial starch and passage to the intestines:

$$DC_s = ScTC_s + StCs + HaCs + HcCs + CeCs - CsFv - CsMi - CsP$$

Cellulose (and hemicellulose) is handled as such: amount of cellulose present is the integration of cellulose in from the diet, cellulose contained in large particles, cellulose contained in small particles, and cellulose passage to the intestine:

$$DCe = CeIn + LpCeCe - SpCeCs - CeP$$

All cellulose in from the diet is allotted to large particles based on the particle size factor:  $CeIn = RCeIn * PSF$ ; large particle cellulose is released based on a degradation rate which can be changed:  $LpCeCe = LpSp * fLpCe$ , where  $LpSp = KLpSp$ . Cellulose lysis to soluble carbohydrate is based on rate which can be set by the user and can also be affected by feed fat:  $SpCeCs = KCeCs * (1 - (fdfat/fdLi * KfatHb)) * Ce$ . Undigested cellulose can pass to the intestine:  $CeP = KSPP * Ce$  based on small particle passage rate (it can be further fermented in the large intestine).

Microbial population is a function of microbial growth and passage rate:

$$DMi = MiG - MiP.$$

Microbial growth is a function of ATP available, which is supplied directly by fermentation of soluble carbohydrate, amino acids or organic acids based on known stoichiometry and accounting for the amount of ATP used for microbial maintenance.

Microbial growth:

$$MiG = ATPG * YATP,$$

$$ATPG = ATPF - ATPM,$$

$$ATPF = CsFv * CsFvAT + RAaFv * AaFvAT + 0.76 * FDNnAm * AaFvAT + RLAFV * LAFVAT.$$

This latter equation describes the sum ATP formed from fermentation of soluble carbohydrate, amino acids, ammonia and lactate. Thus the model strives to meet the basic principles of modeling: to describe the system as closely as possible to reality, limited by what can be validated with present data.

Model Descriptions of Body Processes

Carbohydrate in the body which is metabolized for energy (or to make fat or lactose) eventually is converted to triose phosphates or glucose, or is used through the same metabolic pathways so for simplicities sake we can aggregate a lot of this. So for glucose use in the body as an example:

$$GI = upGI + AaGI1 + PrGI1 + LaGI1 + GyGI1 - GILm - GIHyF - GIHyV - GITpF - GITpV - GILaB - GICd.$$

We sum the uptake of glucose, gluconeogenesis from amino acids (Aa), propionate (Pr), lactate (La) and glycerol (Gy) and the use of glucose for lactose (Lm), triose phosphates (Tp) [in the viscera (V) or body (B)], that used to make pentose phosphates (NADPH<sub>2</sub>, Hy), lactate and that oxidized to carbon dioxide (Cd). Glucose conversion to lactose is a function of the maximal capacity of the gland, which can be set by the user (Uenz are udder enzymes and KMinh is a function of decay of the lactation curve), the sensitivity for glucose, the amino acid supply (to represent beta-lactalbumin), and the concentrations (availability) of glucose and amino acids:

$$GILm = VGILm * Uenz * Kminh / (1.0 + KGILm/cGI + KAaLm/cAa).$$

Thus, as glucose is used by the udder the concentration of glucose changes and this elicits loss from the available glucose. As loss from the pool decreases the pool size (and thus blood glucose concentration) then gluconeogenesis from amino acids increases. This in turn, reduces the amounts of amino acids circulating, and if uptake from the gut cannot maintain the pool, then proteolysis of muscle protein will increase and muscle protein synthesis will increase, allowing maintenance of amino acid supply. Thus, as in the cow, in this model, carbohydrate nutrition cannot be described without invoking amino acid nutrition.

In the transition cow, deficits of glucose are met by two major processes: lipolysis to release free fatty acids that the cow can use for energy and milkfat, and proteolysis of proteins to amino acids for gluconeogenesis. It must be stressed that lipolysis is not only responding to the glucose lack but also to the need by the mammary gland milk fat. So some increase in lipolysis is inevitable. The key source for glucose is amino acids as we wrote about earlier. However, excess lipolysis is undesirable and if we can minimize the glucose lack we can reduce excess lipolysis. This will help reduce fatty liver, ketosis, reduced feed intake and the resultant worse problems.

Supply of glucose also directly affects body fat and protein synthesis. For body fat synthesis, primarily from acetate, we have the aggregate equation:

$$\text{AcTs} = \text{VAcTs} / (1.0 + \text{KAcTs}/\text{cAc} + \text{KGIAcTs} / (\text{Ahor} * \text{cGl}))$$

This shows us the key elements relating to glucose use and therefore nutrition: body fat synthesis is a function of genetics of the cow (V or maximal velocity; and K, sensitivity to substrate (McNamara, 1994); acetate availability (Ac, circulating acetate, primarily from absorbed acetate); and glucose (as direct supplier of reducing equivalent (energy) for fat synthesis); and ‘anabolic hormone’ (which is based on glucose availability). Thus, as glucose availability drops dramatically in early lactation (in relation to demand), as does acetate, the rate of body fat synthesis drops as well, basically to zero for several days (McNamara, 1994). In addition, the ‘anabolic hormone’ of the model is equal to:  $\text{cGl}/\text{rcGl}$ , or glucose concentration divided by reference glucose concentration at energy balance = 0. As glucose drops, so does anabolic hormone (just like insulin) and this further reduces the rate of body fat synthesis. Thus in the model, the increased milk production puts a drain on available glucose and acetate, food intake is not yet sufficient to meet the total needs, and the integrated system reduces the rate of body fat synthesis and increases the rate of body fat loss.

So please do remember that when we discuss and practice ‘nutrition’ we really mean what is happening to the metabolism of the udder and other body tissues. When we design a ration with the right proportions of ingredients and process and feed it to maximize rumen health and feed intake, then the body can support optimal milk production. At the same time, the body (adipose tissue and muscle) is also responding to make up the lack or to store the excess. Nutrition cannot be fully appreciated unless we understand what is happening in the body as a whole.

Where amino acid use per day is the sum of absorbed amino acids, amino acids released from the body (Pb) and from the viscera (Pv) and amino acid use for body protein synthesis (AaPb), visceral protein synthesis, milk protein (Pm), gluconeogenesis (AaGl) saliva and pregnancy. In short, because you are probably tired of all this biochemistry by now, as glucose supply decreases in early lactation, the only major source in addition to propionate absorbed from the gut, is amino acids residing in body proteins. Circulating blood proteins are broken down and the amino acids oxidized and converted to glucose, but that is a tiny percentage of the need. As the body viscera (gastrointestinal organs, liver, and udder) usually grow or at least stay the same size in early lactation, no net glucose can be derived from proteolysis there. Thus that leaves body muscle protein as the major source of amino acids for glucose. Gluconeogenesis was described above and is represented in the published model as a summation of amino acid use:

$$\text{AaGl} = \text{VAaGl} * (\text{EBW}^{**0.75}) / (1.0 + \text{KaaGl} / \text{cAa}),$$

such that as amino acid concentration goes up, glucose synthesis does as well. Simple, right? But this **IS nutrition** in the high-producing dairy cow. When anywhere up to 35 % of the glucose must be supplied, for a period of several days to weeks, from body protein, we must pay attention to it.

## **The next era--genetics, transcriptomics, nutrition, reproduction as a full systems biology approach.**

Now, we must move forward. We have known for 40 years that nutritional processes are controlled by genetically determined characteristics as well as in response to nutritional environment. But for many reasons we have not yet fully integrated genetics, nutrition, reproduction into our management models, and it is past time. We now have basic research tools that are relatively inexpensive and easy to use to ask questions about the control of efficiency in the dairy cow. Use of transcriptomic and other technologies has become a mainstay of biological research in the last few years, and this is good. How can we use the study of transcriptional regulation to improve efficiency of animal production? We can do it in an ordered systems biology approach that focuses on why and how cells regulate energy and N use, and study this within practical situations applicable on farms. Using existing metabolic models we can design experiments specifically to integrate new data from transcriptional arrays into models that describe nutrient use in farm animals. This approach can focus our research to make faster and large advances in efficiency, and show directly how this can be applied on the farms. Where do transcriptomics fit in the system of research in control of animal production?

I think a series of quotes from Cornish-Bowden (2005) helps put 'systems biology' in perspective:

“The idea of systems biology is not new: as long ago as 1968, the mathematician and engineer Mihajlo Mesarovic regretted that “in spite of considerable interest and efforts, the application of systems theory in biology has not quite lived up to expectation”. But what of systems biology today? Does it now look more likely to lead to the expected benefits?”

“In the 1950s the geneticist and biochemist Henrik Kacser was already urging biologists to take systems seriously: “The problem is ... the investigation of systems, i.e. components related or organized in a specific way. The properties of a system are in fact ‘more’ than (or different from) the sum of the properties of its components, a fact often overlooked in zealous attempts to demonstrate ‘additivity’ of certain phenomenon. It is with these ‘systemic properties’ that we shall be mainly concerned...”

“In attempting to define systems biology, Olaf Wolkenhauer (University of Rostock, Germany) emphasized the need for a shift in focus away from molecular characterization towards understanding functional activity.”

Recently we used the systems modeling approach to ask the question of “What patterns of metabolic flux exist in dairy cattle of varying genetic merit and intakes?” Also “Related to that flux, which genes are changing transcription in the adipose tissue?” This was in direct, if delayed, response to a challenge laid out years earlier by Baldwin (Baldwin et al., 1980): “when considerable biological variation exists, opportunities for improvement are embedded within the variation...” and: “...observed efficiencies



considerably below theoretical are also observed. This raises two important questions: (1) Could we learn to identify animals that are capable of attaining maximum efficiencies and based on genetic selection improve the average efficiency of animal production? (2) If we knew exactly what types of unfortunate metabolic decisions that the less efficient animals were making, could we manipulate the metabolism of those animals such that their efficiencies would approach those of the best animals?" Given that these comments were made in 1980, in retrospect it is clear that many scientists have since then done exactly that (SNP's, QTL's...) but many have not taken on the task of integrating the gene with the metabolism.

Thus, in order to do just that, data were collected from several studies done at WSU, with 1st to 4th parity cows, from 28 d prepartum to 120 DIM and included total food intake, nutrient composition of intake, milk and component output, body fat and protein, and transcript levels for several key metabolic control proteins and enzymes expressed in adipose tissue. These cows were all on similar (if not the same diets), from the same herd, spread over several years. The Molly model (Baldwin, 1995; McNamara and Baldwin, 2000); was used to simulate the metabolism of each cow (n = 126 from 3 studies) from 0 to 120 DIM. Input variables included daily feed intake and chemical composition, initial body weight, fat and protein content. Outputs included all milk components, and pathway fluxes for lipid and glucose in mammary, body and visceral energy and protein, and changes in body fat and protein. Simulations were then continued until d 305 to predict potential overall efficiency. Body fat, body and visceral protein all varied ( $P < 0.05$ ) in their daily flux, with genetic merit (predicted transmitting ability for milk) and total net energy absorbed being the greatest contributors to variance. Means (ranges) for all cows were 112 (89 to 139) Mcal/d for intake energy, 32.3 (19.9, 41.9) for maintenance; -0.51 (-1.74, -0.015) for change in body energy; and 0.843 (0.826, 0.862) for net energy efficiency (milk energy/ (energy absorbed – maintenance E)). The model predicted response to dietary energy, dietary fiber and dietary protein content within 1 standard deviation of the observed ( $P < 0.05$ ).

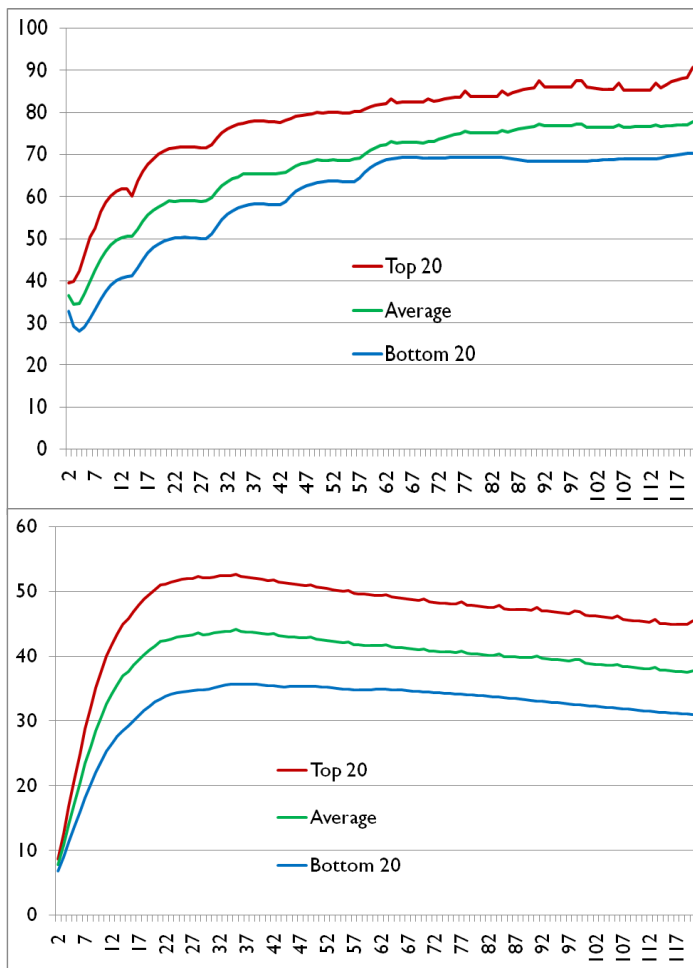
The interesting finding was that variations in maintenance functions (tissue metabolism) affected overall efficiency while mammary efficiency approached the theoretical maxima, as Baldwin predicted 40 years ago (Table 1). Even within a herd of cows quite similar genetically, there was a range of milk productions and feed intakes (as expected) but in fact the variation in metabolic pathways in the adipose, muscle and liver were even more striking). Even within a herd of similar cows on the same diet, use of energy for metabolic functions can vary 100 % between animals. Why? There remains significant undefined variation in metabolism that defines the summative energy efficiencies. Studying energy efficiency with a goal of making all cows more efficient must be done in the context of understanding the system where it is controlled--at the pathway level in individual organs.

**Table 1. Energetic efficiencies of dairy cattle in early lactation as simulated in Molly from actual data.**

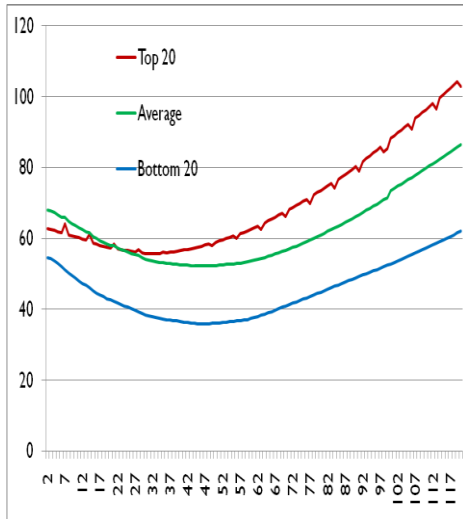
Efficiency measure	Milk Energy,	Milk Energy,	Milk Energy,	Mammary	
		%, GEI	%, ABSE	% ABSE + BE	efficiency <sup>1</sup>
Top 20 %	26%	43%	44%	84%	
Average		23%	38%	38%	84%
Lower 20 %	21%	34%	34%	85%	
SD		2%	3%	3%	1%

1. Last measure is milk energy production divided by mammary energy uptake. This is the thermodynamic maximal value.

Similarly to energy use, N use varied as well. Nitrogen intake was 0.66 (0.52, 0.81) kg/d; milk N, 0.21 kg/d (.16, .27), change in body N, -0.016 (-0.06, -0.004), N in urea was 0.31 (.26, .37) and N balance was -0.018 (-0.032, -0.008). Animals varied in non-mammary E and N use, and the model identified ( $P < 0.05$ ) differences in E and N in the 20 % top versus 20 % lowest efficient cows that start a quantitative metabolic control map of efficiency.



The figures demonstrate the point: even in a fairly homogenous group of cows eating the same rations, there is tremendous variation in milk production, feed intake and associated tissue metabolism. The first figure shows 3 terciles of energy absorption (ME, megacal/d), the second figure the daily milk production in those same groups. The third figure on the next pages shows body fat. So the most efficient cows ate more, gave more milk AND restored body fat faster—who would have thought that from dogma and the approach of just looking a one piece of the system at the time. Yet the efficiency of energy use by the mammary gland is the same. So what does this mean in the system of the cow? We can increase efficiency, but we cannot change the laws of



thermodynamics. More seriously, we cannot increase efficiency through means that would disrupt the normal cell system.

Thus we must pinpoint the critical control mechanisms that vary metabolic rates in the liver, gut tissues, muscle and fat; and ask the questions: Can these efficiencies be changed? And more importantly “Can they be changed without altering the basic system to the detriment of the animal?” The answer is, of course, yes, because we can identify those animals that are the most efficient utilizers of nutrients and identify their control points.

In order to help answer the questions of control, in 2007, we reported for the first time the level of transcripts for HSL, Perilipin and the B1, B2, and B3 adrenergic receptors. In that study of approximately 20 animals, all of these transcripts increased in amount during lactation, with a peak around 90 DIM, which is when milk production was highest. This indicated a role for increased transcription in control of overall lipolytic activity, but the pattern was more subtle. The increase did not peak until lactation also did, suggesting that this is not an ‘early response’ to the negative energy balance and increased milk production of early lactation. Rather, this seems to be a secondary response over time.

When we asked the question of proportional control through multiple regressions, we began to learn more about the system relating transcriptional control with lipolysis. When we regressed the expression of the B2-adrenergic receptor on BW, BCS and empty body fat, we could define only about 10 % of the variation. When we focused the regression comparing B2AR transcript on the maximally stimulated rate of lipolysis, again, only about 10 % of the variation could be defined. This likely suggests that in fact, only about 10 % of the control of lipolysis during lactation can be attributed to an increase in message for this receptor. Given all the other levels of control on lipolysis, and that in fact amount of adrenergic receptor is controlled in a loop of increased stimulation, reduced receptor activity, and attenuation of response (a ‘governor’, if you will to avoid rapid mobilization).

From this same study we then conducted an analysis of the gene transcriptome in bovine adipose tissue during the transition from pregnancy to lactation (Sumner et al. 2008a, b). Animals averaged 29.8 (SEM = 1.3 kg/d of milk for the first 60 DIM (range 18.6 to 44.8 kg/d). They lost 42.6 kg of BW (SEM 8.4, range +9.1 to -113.6) and 0.38 BCS units (SEM 0.10, range 0 to -1.0) from 0 to 14 DIM. This is a normal range for dairy cattle, housed and fed alike and gives a glimpse of the yet unknown effects of genetic variance in a similar population.

We obtained adipose tissue by biopsy at 30 d prepartum and 14 d postpartum and extracted the RNA. This was hybridized to the Affymetrix Genechip® Bovine Genome

Array. Anabolic pathway genes (Table 2) decreased ( $P < 0.05$ ), including (mean (% change), (SEM)): steroyl response element binding protein, -25.1, (6.2); glucose transport 1, - 57.3 (14.1); thyroid hormone receptor spot 14, -30.8 (7.4); lipoprotein lipase, -48.4 (7.7) and AcCoA Carboxylase, -60.6 (13.0). The regression of transcript change on milk production was 0.18 for AcCoA carb and 0.26 for ATP-CL ( $P < 0.05$ ). Lipolytic control elements increased, with much variation among animals, including Ca channel subunit 338 % (203); B2AR 52.0 (8.8); PKC receptor 10.1 (2.6) and HSL mRNA 23.0 (17.9). The regression of transcript change on milk production was 0.30 and 0.25 for B2AR and HSL mRNA. These latter regressions explain somewhat more of the variation than the ones for HSL and B2AR in lipolysis, which is intriguing. These results lead us to conduct further more in depth studies to integrate transcriptional control into the metabolic model.

A total of 48 cows were grouped by their sire PTAM: High Genetic (PTAM = 870 kg), or Low Genetic (PTAM = 378), and half of each group was fed either to requirements (NE) or to 90% of energy requirements (LE). Other components were fed to requirements. Feed intake from 21 to 1 d prepartum was 13.6 (NE) and 12.7 kg (LE) DMI/d (SE = 1.5). From 1 to 56 DIM it was 21.2 and 17.4 kg/d (SE = 1.4). Milk production was 36.1 and 33.3 kg/d for HG and LG cows from 27 to 56 DIM ( $P < 0.05$ ). Adipose tissue biopsies at -21, -7, 7, 28 and 56 days around parturition were used to measure lipolysis, lipogenesis and gene expression. Rates of lipogenesis were lower during lactation and lower in LE cows while lipolysis rates were higher for both conditions ( $P < 0.05$ ). The mRNA expression of the beta-2 adrenergic receptor, hormone sensitive lipase and the co-lipase, perilipin, was several-fold higher ( $P < 0.05$ ) in animals on restricted energy. The mRNA for caveolin-1 and caveolin-2 decreased 20 to 40 % ( $P < 0.05$ ) in lactation consistent with the increase in lipolysis and HSL message. The gene expression array showed coordinated decreases in genes regulation lipogenesis (TRPSP14, -26 %; AcCoCarb, -76 %; LPL, -57 %; ATP-Citrate Lyase, -22 % as examples) and no change or moderate increases in those controlling lipolysis (Table 2).

**Table 2. Genes coding for metabolic control in adipose tissue of lactating dairy cattle.****Genes Coding for anabolism**

Gene	GenBank	<u>-7</u>	<u>7</u>	<u>28</u>	<u>d 28 / d -7</u>	P
LPL	BG688620	4045	2229	1552	-57%	0.00
FABP5	NM_174315.2	4378	4265	3075	-22%	0.88
GLUT4	NM_174604.1	49	37	36	-26%	0.53
THIHP	CK848521	2679	1148	625	-71%	0.01
ATP CL	CB433477	471	401	351	-22%	0.00
AcCoACarb	NM_174224.2	162	55	39	-73%	0.00
AcCoACarb	BE751005	100	31	21	-76%	0.00

**Genes Coding for catabolism**

Gene	GenBank	<u>-7</u>	<u>7</u>	<u>28</u>	<u>d 28 / d -7</u>	P
HSL, mRNA	CK769629	39	31	53	-4%	0.28
HSL	BM967863	77	60	80	19%	0.14
b2AR	NM_174231.1	155	138	92	-32%	0.02
CAV1mRNA		1111	905	977	-14%	0.06
CAV1		3549	2813	2714	-23%	0.90
CAV2a		625	481	395	-33%	0.65
CAV 2		834	480	423	-47%	0.04
CAV 2		156	77	74	-52%	0.05

Samples biopsied at times around calving as indicated. Results are signals from the Bovine Affymetrix Gene Array, normalized to an average signal strength of 125.

Further we were able to run regressions of gene expression on milk production. For the genes listed, in parentheses are the regression coefficients for gene expression versus milk production in the first month of lactation: GLUT1 (0.34); IGFBP3 (0.67); THRP 12 (0.38); LPL (0.18); leptin (0.31). All of these genes controlling anabolic reactions were negatively related with milk production. These regression coefficients give us some mathematical insight into how much control might be exerted on the anabolic pathways by gene expression. There was little relation between milk production and lipolytic control genes, again suggesting that most control on lipolysis is physiological.

So, here now we can use a systems biology approach, based in sound biology, and use the model to ask deeper questions about control of the system. Changes in gene expression alter the maximal velocity of lipogenesis and lipolysis. These changes measured in the cows can be used to alter the maximal velocity parameters of lipogenesis and lipolysis in body fat, in direct proportion to the relative change in transcript level, based on the principle that mRNA abundance directly relates to enzyme

concentration, but is independent of post-translational modification. We can then add the control by post-translational physiology (already in Molly through anabolic and catabolic hormone control). Integration of these control elements into metabolic models provides the opening to more fully explore the relationships of genotype, phenotype and nutritional environment on the efficiency of dairy cattle.

### **The acceptance of integrative biology is critical**

A major barrier to improvement of models and thus their increased use, remains lack of an accurate description of the phenotype of the animal being modeled, expressed as, for example, gene transcription control, enzyme activity, hormone and receptor kinetics and intracellular signaling. An additional barrier continues to be the thought processes of scientists who are not trained in more complex regulation and theories and are uncomfortable with the ideas or skeptical of the value of integrative biology.

One underlying concept to such integrative work is that the amount and activity of all enzymes and hormones are genetically regulated, from immediate gene transcription and translation, to heritability of variations in hormone and enzyme synthesis and secretion.

The flood of information from the various genome works, and the ability to generate large volumes of transcriptome data from animal studies has renewed calls for more integration of knowledge, including using bio-mathematical approaches. A model or a modeling approach to research may also be defined as an ordered way of describing knowledge of some real complex system. Such models have been useful in practical systems to describe, for example, drug metabolism, biochemical pathways and nutrient requirements. A quantitative description of metabolic transactions is critical to improve understanding and improvement of nutrient requirements, health and longevity. Models of increasing complexity, ever grounded in validated research data, will continue to improve our quantitative understanding. It is this author's experience that information from genomic research can only be understood with the means of complex model systems, a philosophy shared by others (Dawson, 2004).

We have a long way to go. We need a re-invigorated, multi-investigator, multi-disciplinary, integrated approach to solve the present and future problems of reproduction, and specific to the role of nutrigenetics and nutrigenomics for improved reproduction, this research effort will require construction and testing of mechanistic bio-mathematical models. Finally, we need to train students, scientists and professionals in the importance of using integrative biology and bio-mathematical models to identify, solve and prevent reproductive problems.

## TAKE HOME MESSAGE

Our job as University Scientists and Educators is to provide the newest and most useful information and to look at the future, not solve problems of the past.

Challenges in dairy business require integrated solutions, even at the cow level.

Amino acids, glucose and fat integrate to allow those solutions—the cow and proper feeding will allow a prosperous business and keep a clean environment.

Research: University herds and budgets can no longer match commercial herds for experimental units. We need to work together to improve.

### **Find out what is really happening in your herds pertaining to carbohydrate nutrition:**

- ✓ Percentages of Neutral Detergent Fiber, Acid Detergent Fiber and Lignin
- ✓ Percentage of Starch and solubility of starch (similar figures would be non-structural carbohydrates and solubility or degradability)
- ✓ Effective fiber-know the average and distribution of particle length.
- ✓ Amount and balance of amino acids, one good way is using the NRC metabolizable protein approach.
- ✓ Feeding delivery-feed a TMR, twice a day if possible, and keep it pushed up and fresh.
- ✓ Allow sufficient bunk space for easy feeding of all animals.
- ✓ Monitor and estimate intake by using delivery data and occasional weighing of refusals-and monitor sorting.
- ✓ Use a fresh cow group to manage these cows well.
- ✓ Expect and manage for rapid increases in milk production and feed intake in early lactation- a target may be 10 % increase in milk production per day for 14 days for cows and 8 % a day for 18 days for heifers (110 lbs in 20 days, 80 lbs for heifers). This target may be modified to the situation in your herd.
- ✓ Aggressively offer a well-balanced ration in the late dry period (30 days out) to include all of the above and sufficient energy and protein to stimulate the rumen microbes and body systems.
- ✓ Dairy animals do NOT have to have a large reduction in feed intake in the last week if the diet is formulated and fed to stimulate appetite and the rumen, AND if the cow 'requires' the diet—does she have the genetics to require the 'hotter' diet and eat it.
- ✓ Aggressively and preventatively treat fresh cows at all levels of risk. Daily temperature for 10 days, veterinary treatments in consultation with your vet (ECP? Other).
- ✓ Consider feeding some direct gluconeogenic precursors (Calcium Propionate) to all cows, and/or warm drenching with this or other solution (propylene glycol).

- ✓ Our goal here is to prevent the percentage of cows who ‘go off feed’ for whatever reason—for every one cow you prevent a displaced abomasums, ketosis, fatty liver or lost production you save hundreds of dollars.
- ✓ Collect proper dynamic data over months—feed intake, component intake (forage/concentrate), nutrient intake (NDF, ADF, lignin, CP, RUP, UIP, fat, NE). “Book” values are usable if you have a good record of dietary composition and some spot samples or good references (spot sample forage for CP, ADF/NDF).
- ✓ Record milk components, body condition score and weight, health history—individual cows over time if possible, but herd or tank data can be used if you have a long enough period. Think about how many things can change milk test or performance in the short term only. Don’t use a short-term measure to provide a long-term prediction.
- ✓ Share ‘real’ data with scientists so we can provide useful models to help you do your job even better. We don’t need proprietary information—just good data.

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