Healthy Livers Make for Healthy Cows

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Take Home Messages

- Transition cows must exquisitely coordinate their metabolism to meet tremendous increases in nutrient demand during early lactation. These include adaptations in whole-body glucose metabolism and liver-specific adaptations relative to utilization of individual substrates for glucose synthesis.
- Mobilization of nonesterified fatty acids (NEFA) from body fat during the transition period appears to present challenges to the capacity of liver to synthesize glucose, either directly or indirectly through impaired capacity of liver to detoxify ammonia to urea.
- Opportunities to manage metabolism of NEFA and improve metabolic health of transition cows exist both through supply-side manipulations and potentially through nutritional modulation of pathways of fat metabolism within liver.

Introduction

The transition period of the lactation cycle in dairy cattle is characterized by dramatic changes in nutrient demand that necessitate exquisite coordination of metabolism to meet requirements of the mammary gland after calving for energy, glucose, and amino acids. Estimates of demand for glucose, amino acids, fatty acids, and net energy by the pregnant uterus at 250 days of gestation and the lactating mammary gland at 4 days postpartum (Bell, 1995) indicate approximately a tripling of demand for glucose, a doubling of demand for amino acids, and approximately a 5X increase in demand for fatty acids during this timeframe. The cow in turn relies on metabolic controls to enable these changes in nutrient partitioning to occur. As discussed earlier at this conference, the two critical metabolic adaptations underpinning successful transitions to lactation are:

1) The cow alters her glucose metabolism to meet the dramatically increased demand for glucose following calving.

2) The cow mobilizes large amounts of body fat to support lactation, which has important ramifications for liver function and metabolic health.

The liver plays a central role in both of these critical adaptations; therefore, the remainder of this paper will integrate changes that occur in the physiology of the liver in the context of these major metabolic adaptations and provide some insight into "managing metabolism" on commercial dairy farms.

■ Adaptations of Liver Metabolism During the Transition Period

The liver is one of the most metabolically active tissues of the ruminant, utilizing approximately 25% of whole-body oxygen consumption while accounting for only about 2% of body weight (Huntington and Reynolds, 1987). Because essentially all nutrients absorbed from the gastrointestinal tract must pass through it, the liver has a major influence on the quantities and types of nutrients that are supplied to the peripheral tissues for maintenance and productive functions. Because of this place at the crossroads of metabolism, the liver is a logical candidate for regulation during the transition period. Data in Table 1 indicate that liver size does not change greatly during the transition period; however, oxygen uptake, an indicator of metabolic activity is approximately doubled during early lactation compared with the late dry period.

Table 1. Liver mass (wet weight) and oxygen uptake by liver tissue during the transition period (Reynolds et al., 2000a; 2000b).

	Day relative to calving			
	-21	-7	10	22
Liver weight, kg	9.0	8.8	8.8	9.6
	Day relative to calving			
	-19	-11	11	22
Oxygen uptake, moles/d	35.4	38.8	75.8	80.1
Oxygen uptake per unit of tissue, moles/(kg/d)	3.9	4.4	8.6	8.3

Glucose metabolism in the transition cow

Some of this increased metabolic activity is a result of an increase in glucose synthesis by the liver after calving. We have discussed previously in this conference that glucose represents an overriding metabolic demand during the transition period. Calculated glucose supply based upon intestinal absorption

of glucose and synthesis of glucose by the liver from nutrients derived from the diet, is approximately 500 grams per day less than the cow requires during the first three weeks or more postcalving (Figure 1).

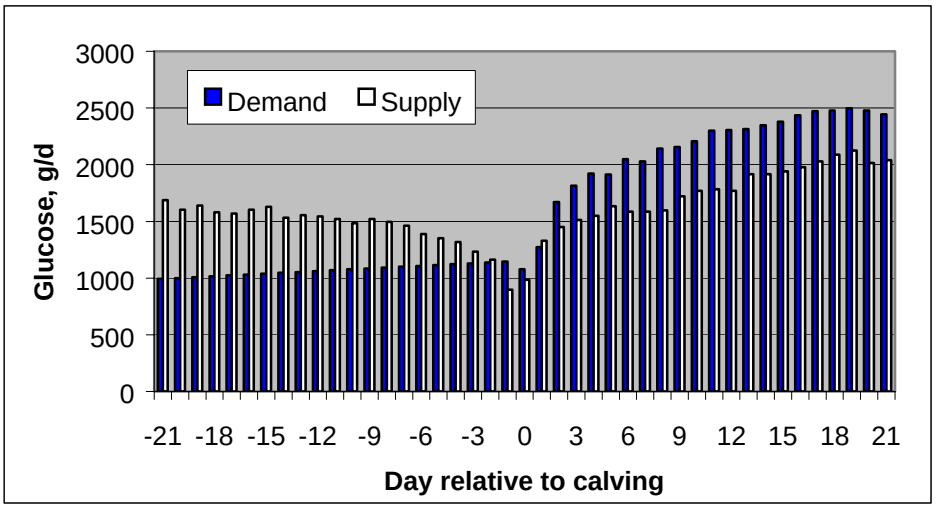

Figure 1. Predicted whole-body glucose demand and supply during the transition period in dairy cows using performance data of Piepenbrink and Overton (2000) and approach of Overton (1998).

Recently, data were obtained using cows surgically fitted with multiple blood sampling catheters in the blood veins draining the gut and liver which enables us to compare our estimates of glucose supply and demand with actual supply (Figure 2). These data indicate that our predicted glucose requirement of approximately 1000 grams per day during the closeup dry period matches reasonably well with both predicted and actual supply of glucose during this timeframe. After calving, the predicted requirement for glucose more than doubles, and predicted supply is less than the calculated demand by 383 and 350 grams per day at 11 and 22 days postpartum, respectively. The actual supply of glucose is much greater than the predicted supply, indicating that sources other than those accounted for by digestible energy intake from the diet are making contributions to liver glucose output during this timeframe. Recent data (Overton et al., 1998) suggest that at least part of the additional glucose is being synthesized from amino acids during early lactation.

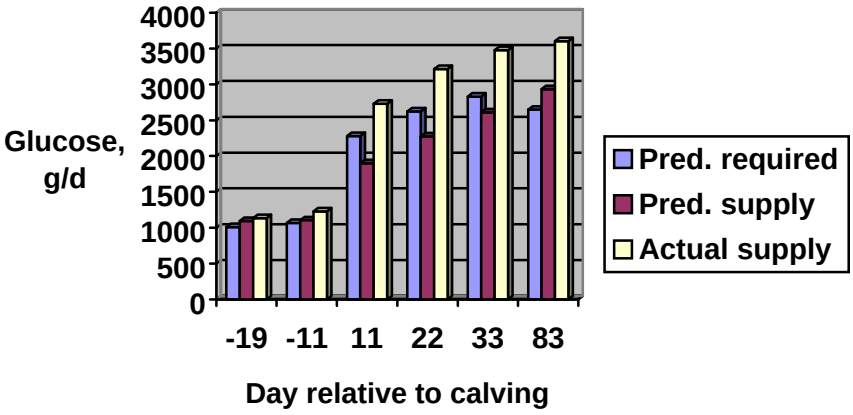

Figure 2. Predicted whole-body glucose requirements compared with predicted and actual supply of glucose by gut and liver during the transition period and early lactation. Data are from Reynolds et al. (2000a). Predictions are as described by Overton (1998).

In summary, liver metabolism in transition dairy cows can be characterized by a dramatic increase in metabolic activity that accompanies the large increase in glucose synthesis during early lactation compared with the late dry period. Available data indicate that nutrient utilization for glucose synthesis by the liver is modulated during this timeframe, and certainly impinge on nutrient requirements of transition cows. Calculations of glucose supply based solely upon energy intake underpredict actual glucose output by liver during early lactation; therefore, other sources such as amino acids must contribute to glucose synthesis during this timeframe.

Mobilization of Body Fat and Ramifications for Liver and Metabolic Health During the Transition Period

Mobilization of body fat results in the release of nonesterified fatty acids (NEFA) into the bloodstream (Figure 3). Available data suggest that the liver takes up NEFA in proportion to their supply (Emery et al., 1992), but typically does not have sufficient capacity to completely dispose of NEFA, either through export back into the bloodstream or burning them for energy (Figure 3). When nutrient intake is insufficient, large amounts of NEFA are released into the blood and the liver begins to accumulate and store the excess NEFA as triglycerides, leading to varying degrees of fatty liver.

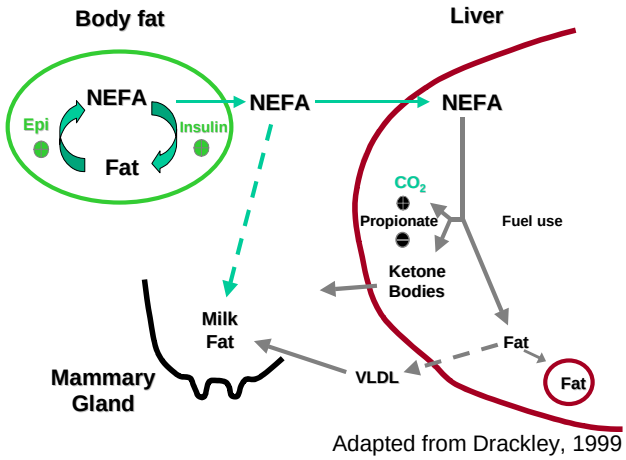

Figure 3. Schematic of metabolism of nonesterified fatty acids (NEFA) in the dairy cow (adapted from Drackley, 1999).

It is likely that a certain amount of triglyceride accumulates in liver of almost all high-producing cows during the first few weeks postpartum. What is uncertain is the threshold at which fat begins to have detrimental effects on liver function. Cadorniga-Valino et al. (1997) demonstrated that fat infiltration into isolated liver cells decreased the livers capacity to synthesize glucose(Figure 4). A followup experiment using a "physiological" mixture of NEFAs determined that fat infiltration did not affect rates of glucose synthesis, but did decrease ureagenic capacity, the process through which ammonia is detoxified to urea (Figure 5; Strang et al., 1998).

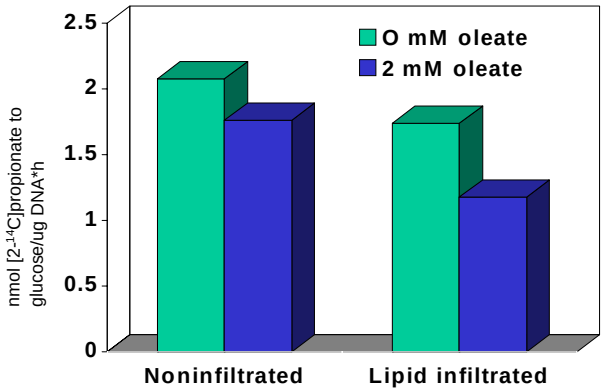

Figure 4. Fat infiltration or concurrent presence of oleate decreases the capacity of liver cells to synthesize glucose from propionate (Cadorniga-Valino et al., 1997).

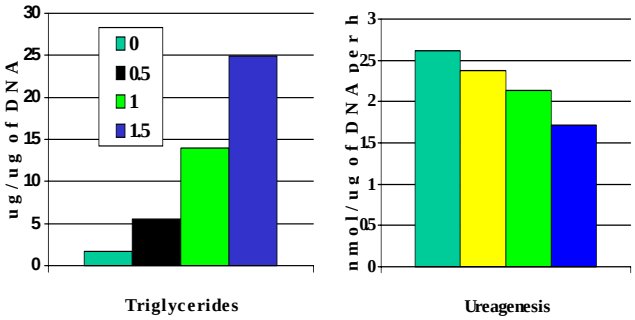

Figure 5. Triglyceride accumulation in liver cells decreases their capacity to detoxify ammonia to urea (Strang et al., 1998).

The implications of this decreased capacity to detoxify ammonia are not clear, but limited evidence suggests that this phenomenon may occur in dairy cows during the transition period. Zhu et al. (2000) determined that concentrations of

ammonia in peripheral blood doubled when liver triglyceride concentrations increased during the first 2 d postpartum (Table 2). Incubation of isolated liver cells with ammonium chloride strongly inhibited their capacity to synthesize glucose from propionate (Figure 6; Overton et al., 1999). Therefore, it is conceivable that inhibition of glucocose synthesis may occur in vivo when triglycerides accumulate in liver; the mechanism perhaps is modulated by ammonia supply to liver. Potential implications for the management of transition dairy cows center around carbohydrate and protein nutrition. As discussed previously, significant quantities of amino acids are needed for synthesis of glucose by the liver. However, excess protein or poorly balanced protein relative to carbohydrate supply may increase the ammonia load on the animal, and thereby reduce the capacity of liver to synthesize glucose.

Table 2. Plasma concentrations of urea and ammonia in peripheral blood around calving and their relation to liver triglyceride (Zhu et al., 2000).

Time relative to calving	Ammonia (μM)	Urea (mg/dl)	Liver TG, %
- 27 days	33.4	5.96	2.58
+ 12 hours	61.1	6.34	. . .
+ 16 hours	64.8	6.08	. . .
+ 22 hours	44.2	5.78	13.10
+ 35 days	28.1	5.68	7.89
Standard error	5.5	0.35	2.81

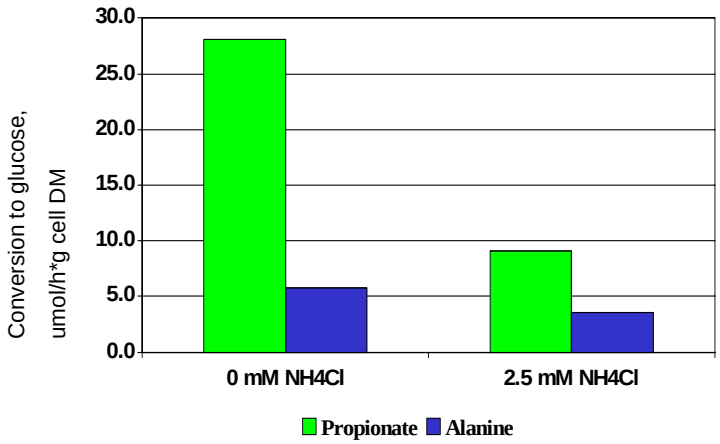

Figure 6. Conversion of [1-^{14}C]propionate and [1-^{14}C]alanine to glucose by isolated liver cells as affected by addition of NH_4Cl in vitro (Overton et al., 1999).

■ Strategies To Manage NEFA In Transition Cows

Management of NEFA during the transition period is an important factor influencing liver health, the capacity of liver to make glucose, and subsequently the incidence of production and metabolic disorders in transition cows. The two primary approaches that can be taken are:

1) decrease the supply of NEFA to liver through diet and feeding management (perhaps use of glucogenic supplements)

2) optimize capacity of liver to dispose of excess NEFA either by burning them for fuel or exporting them back into the bloodstream as triglycerides in very low density lipoproteins (VLDL)

Good closeup and fresh cow nutritional programs, combined with excellent feeding management to achieve high levels of dry matter intake throughout the transition period, achieves 80 to 90% of the potential of the first strategy and should always be the first area of focus for management. Supplements that can be used by the liver to make glucose, such as propylene glycol are effective at decreasing concentrations of NEFA and B-hydroxybutyrate (the predominant ketone body found in blood); however, propylene glycol must be drenched in order to be effective and thus presents both cost and labor challenges.

Even when the first strategy is in place on individual dairy farms, we believe that there are opportunities to further improve liver health by employing nutritional strategies to optimize the capacity of liver to dispose of NEFA rather than accumulate them as fat in liver tissue. As mentioned above, the two disposal routes of NEFA from liver involve burning them for fuel and exporting them back into the blood as triglycerides in very low density lipoproteins (VLDL; Figure 3). It is beyond the scope of this paper to review the complex biochemistry underlying each of these pathways. The reader is referred to reviews by Drackley (1999), Gruffat et al. (1996), and Overton and Piepenbrink (1999) if more detail on the biochemistry is desired.

Evidence from metabolic incubations conducted with liver slices in vitro indicated that use of NEFA for fuel is sensitive to carnitine supply. Carnitine is a quasi-vitamin that is required for transport of NEFA into the mitochondria where they are burned. Carnitine can be supplied through the diet or synthesized in the cow from methionine and lysine, typically considered to be the two most limiting amino acids for milk synthesis in the cow. Experiments evaluating whether liver use of NEFA for fuel can be improved by increasing dietary supplies of carnitine or methionine and lysine have not been conducted.

The second pathway that has potential for nutritional manipulation is export of NEFA as VLDL. Ruminant capacity for synthesis and secretion of VLDL is much lower than nonruminant species. The pathway of VLDL synthesis is complicated, and involves a number of different metabolites that must be present in order to successfully synthesize and secrete a VLDL particle into the blood. French workers (see review by Gruffat et al., 1996) have focused on factors affecting synthesis of the protein called apolipoprotein B100 (apo B), which is required for stabilization of the VLDL particle. They have determined that infusing methionine and lysine into the cow increases release of VLDL by liver, and they have evidence suggesting that the mechanism is increased synthesis of apo B.

Chickens and some other nonruminant species develop fatty liver if they are fed diets devoid of the quasi-vitamin choline. Choline (as phosphatidylcholine) is required as a neurotransmitter and as a component of cell membranes. Phosphatidylcholine is also required in these species for synthesis and secretion of VLDL. Thus, we were interested in determining whether the pathways of NEFA metabolism in liver of the transition cow are sensitive to choline supply.

Ruminants derive choline from microbial synthesis in the rumen. Because choline, fed either as choline chloride or as naturally occurring choline in feedstuffs, is rapidly degraded in the rumen, choline supply to the ruminant can only be meaningfully increased if the choline is fed in a rumen-stable form. We recently completed an experiment to determine whether supplementing rumen-stable choline to diets of transition cows affects liver disposal of NEFA and in

turn decreases triglyceride accumulation in liver tissue (Piepenbrink and Overton, 2000). Samples of liver tissue were obtained via biopsy at 21 days before expected calving and on days 1 and 21 postpartum. Conversion of [1-^{14}C]palmitate to CO_2 (indicator of use of NEFA for fuel) was not affected by choline supplementation; however, the rate of conversion of [1-^{14}C]palmitate to stored esterified products (index of rate of accumulation of NEFA as fat within liver tissue) decreased linearly with choline supplementation (Figure 7). Triglyceride concentration of liver tended to decrease linearly with choline supplementation, and the concentration of glycogen in liver increased linearly with choline supplementation during the transition period (Table 3). Arithmetic derivation of the ratio of triglyceride to glycogen in liver based upon the least squares means for each parameter indicates that this ratio decreased as the amount of choline supplemented to the diet increased. This ratio has been implicated as an indicator of susceptibility to clinical ketosis (Drackley et al., 1992); therefore, our data would suggest that cows fed diets supplemented with choline during the transition period were less susceptible to developing clinical ketosis compared with the controls. In summary, these data suggest indirectly that choline supplementation may modulate the capacity of liver to export NEFA as triglycerides in VLDL. We currently are conducting followup experiments to determine more directly whether the VLDL export pathway is sensitive to supplies of choline and other related compounds such as methionine and its analog.

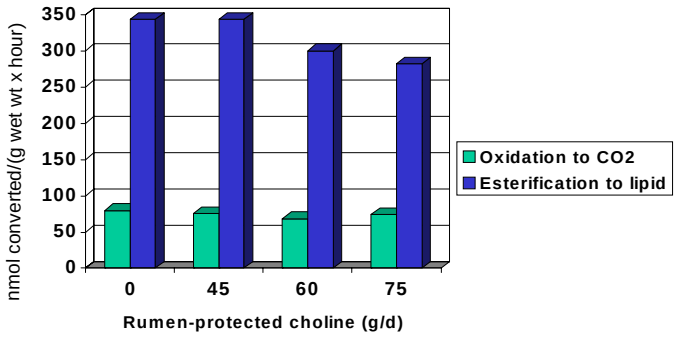

Figure 7. Conversion of [1-^{14}C]palmitate to CO_2 and esterified products by liver slices from cows fed increasing amounts of rumen-protected choline (Piepenbrink and Overton, 2000).

Table 3. Composition of liver samples obtained from cows consuming different amounts of rumen-protected choline from 21 days prepartum through 63 days postpartum (Piepenbrink and Overton, 2000).

	Rumen-protected choline, g/d					
Item	**0**	**45**	**60**	**75**	**SE**	**Effect**
Triglyceride, % wet weight	15.6	14.9	13.2	11.4	2.21	Linear trend of choline supply
Glycogen, % wet weight	0.79	0.81	1.12	1.40	0.21	Linear effect of choline supply
Triglyceride: Glycogen	19.7	18.4	11.8	8.2		

■ Implications

Research conducted during the past several years has resulted in greater understanding of the metabolic adaptations that must occur in liver if cows are to successfully transition to lactation. Recent evidence indicates that strategies can be employed to optimize liver fatty acid metabolism and in turn improve metabolic health of transition cows. These strategies will be increasingly deployed to the dairy industry in nutritional programs for transition cows.

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