

UPDATE ON RUMEN-PROTECTED CHOLINE IN DAIRY COW NUTRITION

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Choline is a quasi-vitamin that has a variety of functions in mammalian metabolism. Its major functions are as the predominant phospholipid contained in the membranes of all cells in the body (as phosphatidylcholine), a component of the neurotransmitter acetylcholine, and as a direct precursor to betaine in methyl metabolism. It is beyond the scope of this paper to review these functions in depth; however, excellent reviews have been published within the past several years that provide substantive overviews of choline metabolism with orientation to the dairy cow (Donkin, 2002; Pinotti et al., 2002). The purpose of this paper is to provide a focused review on the potential for application of rumen-protected choline during the transition period of dairy cows with the end goal of improving transition period success. Furthermore, I will review recent information on the role of choline in reproductive performance. Finally, I will comment on the future of choline as a nutrient in dairy cattle nutrition.

CHOLINE FOR COWS – THE EARLY DAYS...

Early application of choline within dairy cattle nutrition focused on its role in lipid metabolism, similar to its potential today as will be discussed below. Phosphatidylcholine is required for synthesis and release of very low density lipoproteins (VLDL) by liver. Choline deficiency in rats has been demonstrated to result in a six-fold increase in liver triglyceride content (Yao and Vance, 1990). Incubation of hepatocytes isolated from rats fed a diet deficient in choline with either choline or methionine in vitro increased concentrations of phosphatidylcholine in liver and release of VLDL (Yao and Vance, 1988). Methionine contributes to synthesis of choline (Emmanuel and Kennelly, 1984; Sharma and Erdman, 1988a); therefore, it cannot be determined whether the methionine effect measured in their experiment was the result of increasing choline supply or a direct effect of methionine through apolipoprotein B100.

The first series of experiments investigating feeding choline to lactating cows was conducted mostly by Dr. Rich Erdman's group at the University of Maryland. Their focus was primarily to determine whether choline supply potentially limited milk fat synthesis in cows. In their first pair of experiments (Erdman et al., 1984), they determined that there was some increase, albeit variable, in milk fat synthesis by adding choline chloride in an unprotected form to low forage diets. In a second pair of experiments (Atkins et al., 1988), they determined that cows fed choline chloride in an unprotected form beginning four to six weeks postcalving did not affect yield of milk and fat-corrected milk; milk fat percentage and yield tended to increase with choline supplementation. Intraruminal dosing of approximately 30 g/d of choline chloride did not affect flow of choline to the duodenum of steers (Atkins et al., 1988). Subsequent

experiments demonstrated substantial degradation of choline from various feed and synthetic supplements in an in vitro system using rumen fluid (Sharma and Erdman, 1989b) and duodenal increases of less than 1.5 g/d when more than 320 g/d of choline were fed (Sharma and Erdman, 1988b). Collectively, data from these experiments mandate that meaningful increases in choline supply to the tissues of the cow requires supplementation in a rumen-protected form.

Cows fed low forage diets (30% or 40% of DM corn silage as sole forage source) and abomasally-infused with choline demonstrated reasonably consistent increases in milk yield and milk fat synthesis compared to controls or cows fed dietary choline in an unprotected form (Sharma and Erdman, 1989a). In midlactation cows fed more typical proportions of forage in the diet, abomasal infusion of choline did not affect cow performance or circulating concentrations of triglycerides within triglyceride-rich lipoproteins; however, abomasal infusion of soy lecithin increased both circulating concentrations of triglycerides within triglyceride-rich lipoproteins and milk fat synthesis (Grummer et al., 1987). In the first experiments involving feeding choline in a rumen-protected form, Erdman and Sharma (1991) reported that milk production of cows generally increased up to 0.24% choline in the diet, with a magnitude of increase of 4 to 6 lb/d.

CHOLINE AND THE TRANSITION COW

During the past 10 to 15 years, nutrition and management of the transition cow has become a focal point for research, in part because of the recognition of the tremendous changes in nutrient demand that require exquisite coordination of metabolism (Grummer, 1993; Bell, 1995; Drackley, 1999; Drackley et al., 2001; Overton and Waldron, 2004). Failure of metabolic systems to successfully coordinate in support of lactation leads to the occurrence of metabolic disorders, decreased productivity, and compromised profitability on commercial dairy farms.

One of the major metabolic adaptations relates to mobilization of body reserves, particularly body fat stores, in support of the increased energetic demands during early lactation paired with insufficient energy intake. This mobilization of body fat occurs through release of NEFA into the bloodstream (Figure 1). These NEFA are used for energy by body tissues and as precursors for synthesis of milk fat; however, available data suggest that the liver takes up NEFA in proportion to their supply (Pullen et al., 1989). Unfortunately, the liver typically does not have sufficient capacity to completely dispose of NEFA through export into the blood or catabolism for energy (Figure 1), and thus transition cows are predisposed to accumulate triglycerides in the liver tissue (Emery et al., 1992). The primary consequence of this triglyceride accumulation appears to be impaired liver function, including decreased capacity for ureagenesis and gluconeogenesis (Cadorniga-Valino et al., 1997; Strang et al., 1998).

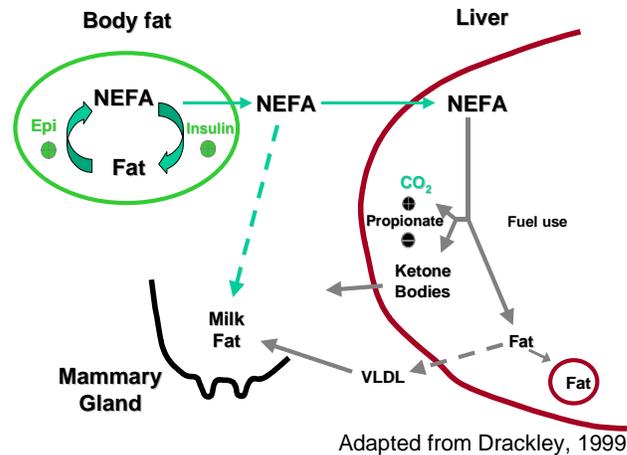


Figure 1. Schematic of metabolism of nonesterified fatty acids (NEFA) in the dairy cow (adapted from Drackley, 1999).

Our research group has worked for the past several years under the guiding principle that management of NEFA during the transition period is an important factor influencing liver health, the capacity of liver to make glucose, and subsequently production and metabolic disorder incidence 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 if provided as a bolus)
- 2) optimize capacity of liver to dispose of NEFA either by burning them for fuel or exporting them as triglycerides in lipoproteins (VLDL)

Good close-up 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. Glucogenic supplements such as propylene glycol are effective at decreasing concentrations of NEFA and B-hydroxybutyrate (BHBA; the predominant ketone body found in blood); however, propylene glycol must be drenched or fed such that it is consumed as a bolus in order to be effective in decreasing concentrations of NEFA and BHBA (Christensen et al., 1997), and thus presents both cost and labor challenges. The duration of treatment in most experiments reported in the literature ranges from 10 to 40 days per cow. Recently, two experiments have been conducted (Pickett et al., 2003; Stokes and Goff, 2001) that report beneficial effects of drenching propylene glycol beginning on the day of calving and continuing for one or two subsequent days -- these short-term treatments are much more acceptable from a cost and labor standpoint and have more potential for commercial application; however, under well-managed scenarios it is unlikely that routine preventive administration of propylene glycol or other compounds provides added value in terms of increased milk yield or improved metabolic health (Lenkaitis et al., 2003; Visser et al., 2002; Visser et al., 2003). Recently, monensin was approved by FDA for use in diets for dairy cows in

the US. Data from Canada in which monensin was administered in controlled-release capsule form (Duffield et al., 2003) and from the US in which the premix form was topdressed (Vallimont et al., 2001) suggest that monensin supplementation to diets for transition cows will decrease circulating concentrations of NEFA as well.

The potential for choline provided in a rumen-protected form to aid in metabolic adaptation to lactation relates squarely to the second approach described above. Based upon the mode of action (VLDL export) described above for the nonruminant animal, we would hypothesize that providing supplemental choline might enhance export of fat from the liver as VLDL and thus decrease fat accumulation in liver during the transition period.

Hartwell et al. (2000) fed either 0, 6, or 12 g/d of choline in a rumen-protected form to cows from 28 d before expected calving until 120 d of lactation. Additionally, each level of choline was fed in prepartum diets containing either 4.0 or 6.2% RUP (both prepartum diets contained 10% RDP). Milk yield was increased by feeding 12 g/d of choline to cows fed 4.0% RUP prepartum, but feeding 12 g/d of choline decreased milk yield in cows fed 6.2% RUP prepartum. Liver lipid content was decreased by feeding rumen-protected choline when prepartum body condition score was 3.75 or greater and 6.2% RUP. Thus, in this experiment protein content of the basal diet appeared to modulate responses to rumen-protected choline.

Scheer et al. (2002) fed either 0 or 15 g/d of choline in a rumen protected form to cows from 25 d before expected calving until 100 d postcalving. Supplemental choline tended to increase milk yield during the first 100 d of lactation (30.4 vs. 32.8 kg/d). A similar magnitude of increase in milk production (2.9 kg/d) was measured by Pinotti et al. (2003), who fed 20 g/d of choline in a rumen-protected form to cows from 14 d before expected calving until 30 d postpartum. Cows fed choline also had decreased plasma NEFA concentrations and decreased NEFA to cholesterol ratio in plasma on the day of calving. These findings suggest that perhaps the risk of fatty liver was decreased by feeding rumen-protected choline in this study.

Piepenbrink and Overton (2003b) fed either 0, 11.25, 15, or 18.75 g/d of choline in a rumen-protected form to cows from 21 d before expected calving through 63 d postpartum. Feeding choline did not affect dry matter intake, but feeding choline resulted in an average increase of 2.4 kg/d of fat-corrected milk yield. Similar to results of Hartwell et al. (2000), plasma NEFA concentrations were not affected by feeding choline. Additionally, plasma BHBA concentrations were not affected by feeding choline. Although differences in concentrations of triglyceride at 1 and 21 d postpartum were not statistically significant among treatments (Figure 2), the concentration of glycogen in liver increased linearly as increasing amounts of choline were fed (Figure 3). Hepatic capacity to convert radiolabeled palmitate to CO₂ was not affected by treatment (Figure 4); however, hepatic capacity to store radiolabeled palmitate as lipid within tissue slices tended to decrease linearly as cows consumed more choline (Figure 4). Collectively, these data suggest that choline functions in the cow to enhance export of NEFA from liver in VLDL, and it is likely that this metabolic effect underpins the milk

yield responses observed across studies. These results have been supported by data from an experiment conducted recently at the University of Wisconsin (Cooke et al., 2004), in which feeding rumen-protected choline (15 g/d of choline) both decreased both liver triglyceride accumulation during induction of fatty liver through feed restriction and also enhanced triglyceride depletion from liver.

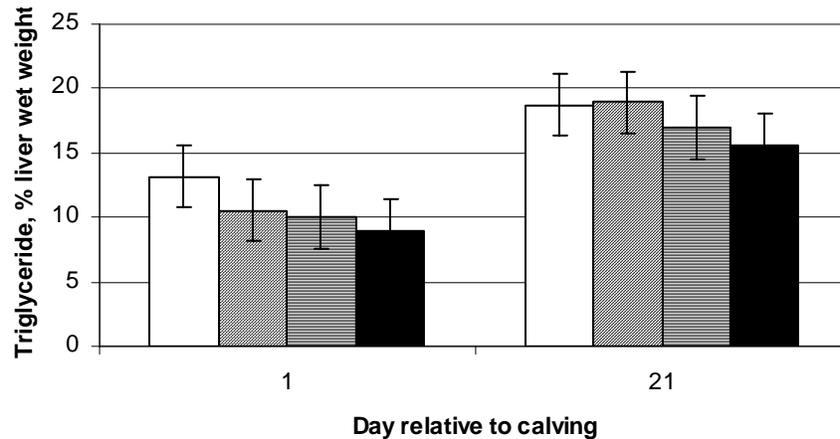


Figure 2. Concentrations of triglyceride in liver from cows fed either 0 g/d (white bars), 45 g/d (diagonal patterned bars), 60 g/d (horizontal patterned bars), or 75 g/d (black bars) of rumen-protected choline (RPC) from 21 d prepartum through 63 d postpartum. From Piepenbrink and Overton (2003b).

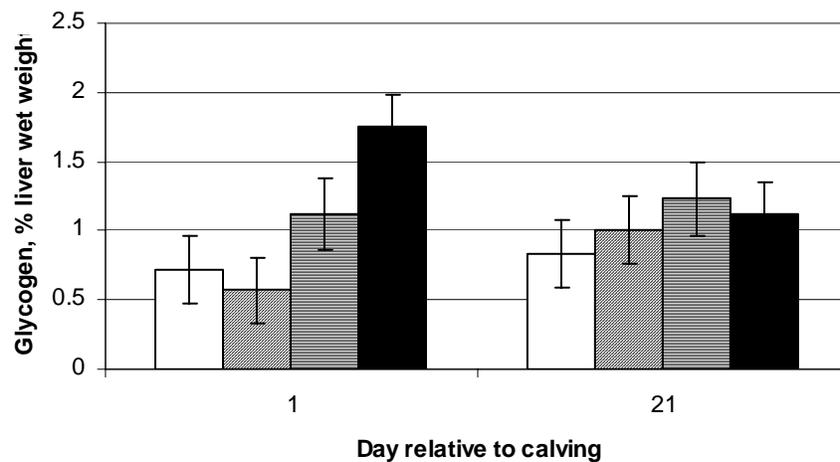


Figure 3. Concentration of glycogen in liver from cows fed either 0 g/d (white bars), 45 g/d (diagonal patterned bars), 60 g/d (horizontal patterned bars), or 75 g/d (black bars) of rumen-protected choline (RPC) from 21 d prepartum through 63 d postpartum. Glycogen content increased linearly ($P < 0.02$) in liver as cows consumed increasing amounts of RPC. From Piepenbrink and Overton (2003b).

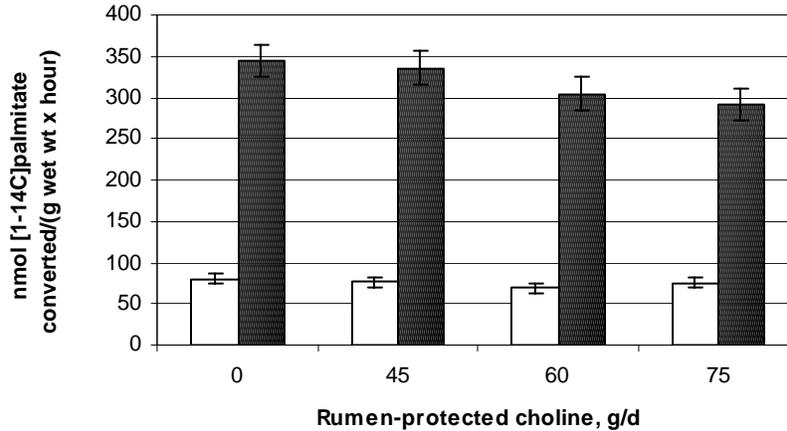


Figure 4. Conversion of [1-¹⁴C]palmitate in liver slices in vitro to CO₂ (white bars) or stored esterified products (black bars). Capacity of liver slices to convert [1-¹⁴C]palmitate to CO₂ was not affected by treatment; however, hepatic capacity to convert [1-¹⁴C]palmitate to stored esterified lipid products tended to decrease linearly ($P < 0.06$) as cows were fed increasing amounts of rumen-protected choline. From Piepenbrink and Overton (2003b).

POTENTIAL FOR CHOLINE TO AFFECT REPRODUCTIVE PERFORMANCE IN DAIRY COWS

During the past several years, increasing attention has focused on the potential relationship of metabolic status during the transition period with subsequent reproductive performance (for a review see Jorritsma et al., 2003). Part of this attention has focused on a negative association between liver triglyceride accumulation and various indices of reproductive function. Although it is unclear whether the relationship between increased liver triglyceride accumulation and impaired reproductive performance is a direct effect related to the processes described above (gluconeogenesis and ureagenesis among others) that apparently are impaired by excessive accumulation of triglycerides or simply a coincidental symptom of the metabolic climate that leads to triglyceride accumulation, evidence for a link between the two continues to grow.

As an example, Marr et al. (2002) categorized cows as ovulatory or nonovulatory based upon whether or not the first dominant follicle detected during early lactation actually ovulated. They determined that cows categorized as ovulatory not only had lower circulating concentrations of NEFA and BHBA and lower liver triglyceride concentrations compared to nonovulatory cows, but that ovulatory cows accumulated less liver triglyceride in proportion to circulating NEFA. This finding suggests that factors specifically related to liver capacity to dispose of NEFA either through oxidation or export may be important for subsequent reproductive function. Interestingly, Piepenbrink and Overton (2003a) reported that triglyceride accumulation was more highly correlated with circulating BHBA than with circulating NEFA, similarly suggesting

the importance of factors associated with NEFA disposal, rather than NEFA concentrations per se, in risk for metabolic disorders such as ketosis.

It is logical, therefore, that compounds such as choline that have been shown to affect liver disposal of NEFA and decrease rates of triglyceride accumulation in liver may impact reproductive performance. Oelrichs et al. (2004a; 2004b) fed dairy cows 15 g/d of choline in a rumen-protected form from 28 d before expected parturition until 100 days in milk. They determined that choline supplementation decreased circulating concentrations of NEFA and BHBA and increased milk yield during early lactation. Furthermore, choline supplementation slightly increased progesterone concentrations during the first synchronized estrous cycle and dramatically improved both conception rate at first service and overall pregnancy rate. Given that choline supplementation continued throughout the first 100 days in milk, it is not known whether these effects on reproductive performance were caused by prior effects on transition cow metabolism as described above or by direct effects of choline on embryo integrity or some other factor related to conception rate and early embryonic loss.

APPLICATION OF RUMEN-PROTECTED CHOLINE IN TRANSITION DIETS

If we conservatively estimate that the economic responses to choline supplementation occur through decreased triglyceride accumulation in liver and subsequently increased milk yield, the most favorable responses to feeding choline to transition cows will occur in cows and herds that are at greater risk for excessive fat accumulation in liver during the transition period. This can be assessed by excessive body condition score loss (> 0.5 units) during early lactation. Furthermore, greater body condition score during the dry period leads to increased loss of body condition score during early lactation (Contreras et al., 2004) and increased fat accumulation in liver (Hartwell et al., 2000), thus herds with overconditioned dry cows (and thus decreased dry matter intakes during the transition period) are likely responders.

Another method to determine whether a herd is a good candidate for a strategy to manage NEFA metabolism such as choline is to determine whether the herd is a high risk herd for subclinical ketosis (Duffield et al., 2002). Although choline itself is not directly antiketogenic, it is likely that high risk herds for subclinical ketosis are mobilizing excessive body condition. Duffield et al. (2002) outlined and evaluated four herd criteria in terms of their ability to predict whether or not a herd was high risk. They found that herds with displaced abomasum incidence greater than or equal to 5%, greater than 40% of cows with a true protein to fat ratio in milk of 0.70 or less at the first monthly test day, or simply 10% or more cows in the herd fat precalving (body condition score greater than 4.0) were highly likely to be at high risk for subclinical ketosis. This can be used as a place to start categorizing herds as likely responders to choline supplementation.

Given the potential for, and current results to support, positive effects of choline supplementation on reproductive performance from the Missouri data, it is becoming apparent that the economic benefits for feeding choline to dairy cows during the

transition period are not confined to specific effects on metabolic health and milk production during early lactation. Although the duration of choline supplementation required to actualize such effects is not known, one could justify moving beyond herds at high risk for excessive mobilization of body condition during early lactation and target either transition period application more broadly and/or feeding for a longer duration than simply the transition and fresh cow groups in high risk herds.

THE NEXT STEP – FORMULATING FOR CHOLINE AS A NUTRIENT

Recommendations for application of choline in the dairy industry to date have been mostly confined to supplementation in a relatively constant amount (15 g/d of choline as choline chloride in a protected form) and to targeted situations as described above. However, choline is a nutrient for which all mammals have a requirement, and this requirement is elevated during late pregnancy and lactation. Furthermore, as described above, part of the methionine requirement of the dairy cow may actually be used to supply a portion of the choline requirement for cows. Clearly, the next step is to develop a system that will allow nutritionists to formulate for choline requirements of the cow in the context of the other major nutrients involved in one-carbon metabolism (methionine and folic acid). Data in dairy cattle that will allow us to do this currently is not available – such data must quantitatively determine both the postabsorptive requirements for these together with the interconversions as related to supplies of these nutrients.

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