

Choline: A Limiting Nutrient for Transition Dairy Cows

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INTRODUCTION

Choline has been shown to be a required nutrient for many animals including rats, mice, dogs, pigs, guinea pigs, chickens, and trout. Choline is often referred to as a vitamin, however, it doesn't fit any of the classical definitions for a vitamin. It is not a co-factor in enzymatic reactions, it can be synthesized endogenously, and it is required in larger amounts than vitamins. The ability to synthesize choline endogenously does not mean it is a dispensable or non-essential nutrient. Deficiency symptoms include suppressed growth rates, renal dysfunction, and development of fatty liver. Choline is crucial for normal function of all cells. The most common form of choline in biological systems is phosphatidylcholine (**PC**), a phospholipid that is a component of all cell membranes and lipoproteins that function to transport lipids through the circulatory system. Choline is a source of methyl groups, therefore, it can spare methionine and have interactions with other nutrients involved in one-carbon metabolism (e.g. folate). Choline is also a component of acetylcholine, an important neurotransmitter.

The National Research Council (**NRC**), 2001 wrote: "The establishment of a choline requirement, either for the lactating dairy cow, or a transition cow in the late dry period and in early lactation, will require more extensive feeding experiments than available at the time of this publication." It has now been 12 years since publication of the last NRC and at this time there has not been an announcement for the formation of a new committee to author the next NRC. That means it will probably be at least 2016 until publication of the 8th revised edition. Since publication of the last NRC, numerous studies have been conducted to examine the effects of feeding ruminally protected choline to dairy cows, particularly as they transition from the dry period to early lactation. In light of new research and because a revised NRC is not on the immediate horizon, it seems appropriate to initiate discussion on whether choline should be considered a required nutrient in dairy diets.

TRANSITION COW AND CHOLINE BIOLOGY

Several studies have shown 50 to 60% of transition cows experience moderate to severe fatty liver (Bobe et al., 2004). These studies have been conducted in numerous countries across different genetic lines of cattle and varying management systems and the data do not represent problem cows or herds. The consistency amongst these studies suggests that development of fatty liver is a "normal" part of the cow's biology. Because fatty liver is a classic deficiency symptom for choline, it is reasonable to question if transition cows are typically deficient in choline.

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At calving there are hormonal changes that trigger an intense period of lipid mobilization from adipose tissue and as a result, blood nonesterified fatty acid (**NEFA**) concentrations typically increase 5- to 10-fold (Grummer, 1993). NEFA remain elevated, although to a lesser extent, during early lactation when cows experience negative energy balance. Blood flow to the liver doubles as a cow transitions from the dry period to lactation (Reynolds et al., 2003). NEFA concentration and blood flow are the two biggest factors affecting how much NEFA is taken up by the liver. As a result, daily fatty acid uptake by the liver increases 13-fold at calving, from approximately 100 to 1300 g/day (Reynolds et al., 2003). Not all of the fatty acids taken up by the liver will be stored and contribute to fatty liver. However, Drackley et al., (2001) estimated that during peak blood NEFA concentration, approximately 600 g might be deposited in 24 hours, which would correspond to an increase in liver fat of 6-7%, by weight. As a reference, fat above 5% in the liver (wet basis) is considered by the veterinary community to be moderate to severe fatty liver. It is important to understand that this dramatic increase in NEFA uptake by the liver is part of the normal biology of transition cows and is not restricted to fat cows, poorly fed cows, or cows housed in suboptimal environments.

The most desirable fate of fatty acids entering the liver would be complete oxidation to provide energy to the liver or reesterification and export as triglyceride from the liver as part of a very low density lipoprotein (**VLDL**). Hepatic oxidation increases approximately 20% during the transition period (Drackley et al., 2001). This increase does not represent a strategic move by the cow's liver to cope with the sudden surge of NEFA uptake at calving. It occurs because the liver becomes metabolically more active. Unfortunately, the increase in oxidation is not sufficient to cope with the increased load of fatty acid being presented to the liver. Research conducted 25 years ago at the University of Wisconsin (Kleppe et al., 1988) and Michigan State University (Pullen et al., 1990) revealed that ruminants have a low capacity to export triglyceride from the liver as VLDL as compared to nonruminants. This and the inability to markedly increase fatty acid oxidation is why transition dairy cattle develop fatty liver when experiencing elevated blood NEFA.

It is now apparent that choline deficiency is a limiting factor for VLDL triglyceride export from the liver. It has been shown in many species, using a wide variety of experimental approaches, that rate of VLDL export is highly related to the rate of hepatic PC synthesis (Cole et al., 2011). Models include monogastrics fed choline deficient diets, isolated hepatocytes cultured in choline and methionine deficient media, and knockout mice for genes involved in PC synthesis (Cole et al., 2011). Interestingly, there is no evidence that synthesis of any other phospholipid is required for hepatic VLDL assembly and secretion. In addition to direct PC synthesis from dietary choline, there is endogenous hepatic synthesis of PC via methylation of phosphatidylethanolamine (**PE**). Sharma and Erdman (1988) demonstrated dietary choline is extensively degraded in the rumen of dairy cows and very little is available to the small intestine for absorption. Choline flow to the duodenum increased less than 2 g/day, even when free choline intake was increased to more than 300 g/d. Therefore, ruminants are more highly dependent than nonruminants on endogenous synthesis of PC from PE. Is endogenous synthesis of PC from PE sufficient during the transition

period or do cows require choline supplementation? The high proportion of transition cows developing moderate to severe fatty liver during the transition period suggests that endogenous synthesis is not sufficient in many cows.

EVIDENCE FOR A CHOLINE DEFICIENCY IN TRANSITION DAIRY COWS

The first piece of evidence that transition cows are deficient in choline is the development of fatty liver during the periparturient period (Grummer, 1993; Bobe et al., 2004). More compelling evidence is the alleviation of fatty liver when supplying cows with choline that is protected from ruminal degradation (Cooke et al., 2007; Zom et al., 2011). Dutch researchers (Goselink et al., 2012) recently demonstrated greater gene expression for microsomal triglyceride transfer protein (**MTTP**) in liver of transition cows supplemented with rumen-protected choline (**RPC**). MTTP is an important protein required for hepatic VLDL synthesis. This provided solid evidence that choline limitation is a causative factor for inadequate fat export out of the liver.

The reduction in liver fat content when feeding transition cows RPC is accompanied by improved health and production. Lima et al. (2011) observed reduced incidences of clinical ketosis, mastitis, and morbidity when feeding RPC from 25 days prepartum to 80 days postpartum. It has been known for years that elevated fat in the liver is associated with poor reproductive performance (Bobe et al., 2004). First service conception rate was increased by feeding RPC in one study (Oelrichs et al., 2004) but not another (Lima et al., 2011). We (Grummer and Crump, unpublished) recently completed a meta-analysis for 13 studies that fed RPC to transition cows (Table 1). Feed stability or evidence of bioavailability of choline source was not a criterion for study selection. Studies were not screened for “soundness” of research. Treatment means and sample size (standard error of the mean) had to be available for the analysis. Ten of the thirteen trials were published in peer-reviewed journals. For studies to be included in this analysis, RPC had to be fed *prior* to calving. Time when RPC supplementation was started varied between 28 to 7 days prior to expected calving. RPC supplementation was terminated anywhere from the day of calving (one study) to 120 days in milk. Response variables included DMI, milk yield, energy corrected milk yield, fat %, protein %, and fat and protein yield. Insufficient data was available for analysis of liver fat or energy-related blood parameters. Analysis revealed a significant increase of 4.9 lb milk/day and 1.6 lb of dry matter intake/day (Table 2; Figure 1). Milk fat and protein yield percentage were not significantly affected by treatment but yields were (Table 2). These studies were conducted in several countries under a variety of management conditions and they did not target problem herds or cows. This implies that benefits to supplementing protected choline can be realized by a wide variety of herds. Alleviating a choline deficiency not only reduces liver fat but also improves parameters that are economically important to dairy producers.

Table 1. Studies used in the Meta-Analysis.

Study	Choline Dose, g/d	Product	Duration	Experimental Units	Parity
Hartwell et al., 2000	0,6,12	Capshure	-21 to 120	24	M
Zom et al., 2011	15	ReaShure	-21 to 42	19	M
Lima et al., 2007 ¹	15	ReaShure	-25 to 80	4 (pen)	M, P
Lima et al., 2007 ¹	15	ReaShure	-22 to 0	5 (pen)	P
Oelrichs et al., 2002 ¹	15	ReaShure	-28 to 100	32	M, P
Zahra et al., 2006	14	ReaShure	-25 to 28	91	M, P
Piepenbrink et al., 2003	11,15, 19	ReaShure	-21 to 63	12	M
Guretzky et al., 2006	15	ReaShure	-21 to 21	21	M
Elek et al., 2008	25/50 Pre/Post	Norcol-25	-25 to 60	16	M, P
Ardalan et al. 2011	14	Col 24	-28 to 70	20	M, P
Pinotti et al. 2003	20	Overcholine 45%	-14 to 30	13	M
Xu et al. 2006 #1	7.5	Not reported	-7 to 21	7	M
Xu et al. 2006 #2	11,22,33	Not reported	-15 to 15	9	M, P

¹Studies have not been published in a peer-reviewed journal. Standard errors were not reported in abstracts but were obtained from the authors.

Table 2. A Meta-analysis of 13 studies examining the effects of feeding RPC to transition cows on dry matter intake and milk.

	Control	RPC	SEd	P =
DM intake, lb/d	39.98	41.60	0.46	0.0042
Milk, lb/d	70.88	77.75	0.75	<.0001
Energy-corrected milk, lb/d	76.87	82.78	1.33	0.0038
Fat yield, lb/d	2.788	3.042	0.086	0.021
Protein yield, lb/d	2.300	2.467	0.053	0.010

Milk Yield (lb/d) by Study

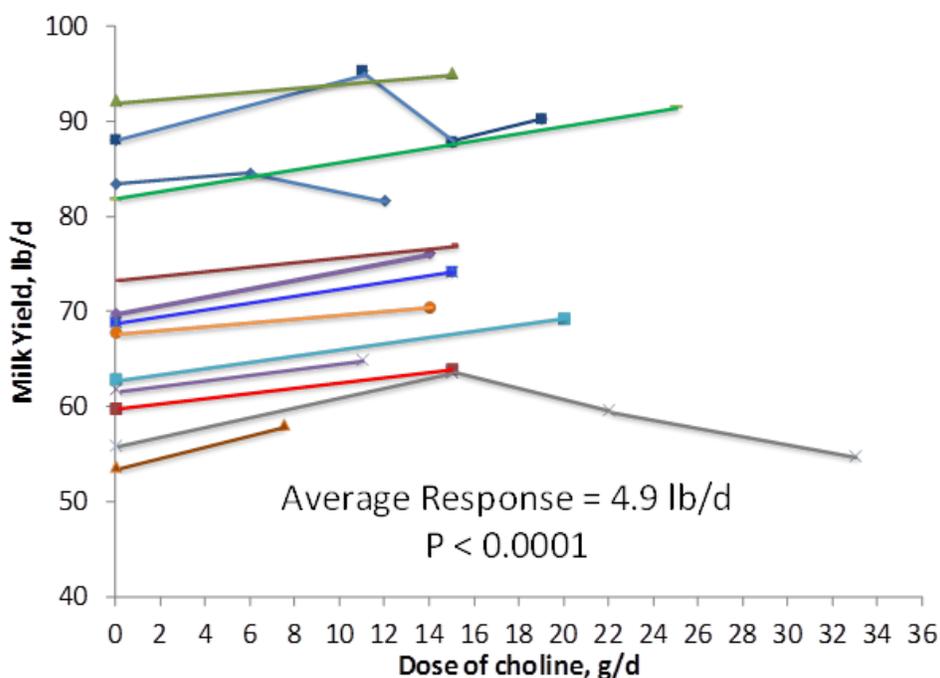


Figure 1. Individual study results from a meta-analysis of 13 transition cow trials that examined the effects of feeding rumen-protected choline (Grummer and Crump, unpublished).

CAN PROTECTED METHIONINE SUBSTITUTE FOR PROTECTED CHOLINE?

Protected methionine has often been suggested as a possible alternative to protected choline for supplementation to transition dairy cows. Methionine and choline both serve as methyl donors. Methionine methyl groups can be used for endogenous synthesis of PC from PE. Therefore, there is a conceptual basis for methionine substitution for choline. Additionally, as an amino acid, methionine is needed for the synthesis of apolipoproteins.

Five feeding trials have been conducted to examine the effects of feeding methionine analogs or protected methionine on liver fat content. Feeding 13 g/d of 2-hydroxy-4-(methylthio)-butanoic acid (**HMB; also referred to methionine-hydroxy-analog or MHA**) did not reduce triglyceride accumulation in the liver of feed restricted dry cows (Bertics and Grummer 1999). Feeding 0, .13, .20% of dry matter as HMB from 21 days prepartum to 84 days postpartum did not affect liver triglyceride at 1 day postpartum and resulted in a tendency ($P < 0.15$) for a quadratic increase in liver triglyceride at 21 day postpartum (Piepenbrink et al., 2004). They also observed a quadratic effect of HMB for increased fat-corrected milk yield providing further indication that the cows were responsive to treatment. The amount of HMB absorbed from the

gastro-intestinal tract and converted to methionine by the liver has not been well established.

Cows fed 0 or 10.5 g methionine/day as Smartamine from 14 days precalving to 105 days postcalving had similar liver total lipid postcalving (Socha, 1994). Liver triglyceride was not measured. Milk protein percentage was increased by treatment indicating that supplementation delivered more methionine to the blood stream. Feeding 9 g Mepron/day precalving and 18 g Mepron/day postcalving increased liver triglyceride ($P=0.02$) but the means from 4 liver biopsies taken over 16 weeks were small and the increase was small (Preynat et al., 2010). Milk protein percentage was increased by feeding Mepron which indicated an improved methionine status. Feeding MetaSmart (.18% of DM) or Smartamine (.07% of DM) from 21 days prepartum until 20 days postpartum did not affect total lipid and triglyceride concentrations in the liver (Osorio et al., 2001). The researchers indicated that the slope of liver total lipid between day 7 and 21 postpartum was different ($P < 0.04$) for cows fed MetaSmart and Smartamine implying that methionine prevented increased lipid accumulation during that time. The justification for this method of analyzing the data was not obvious because the researchers did not indicate that there was a significant time of sampling by treatment interaction. Dry matter intake, milk yield, and fat percentage were increased by methionine supplementation indicating that methionine status was improved (Osorio et al., 2011b). Further examination of the data must wait until a full length report becomes available in a peer-reviewed publication. Considering the five studies conducted to date, there is insufficient evidence to suggest that feeding methionine analogs or protected methionine can replace protected choline for the prevention of fatty liver.

CONCLUSIONS

The time between NRC publications is increasing and when (or if) the next publication will occur is not known. Consequently, discussions outlined in this article become important for providing nutritionists with updates regarding nutrient requirements. Since the last NRC (2001) publication, a significant body of evidence has accumulated to support choline being a required but limiting nutrient in transition cow diets. An analogous situation occurred when the last NRC (2001) committee included a supplemental vitamin E recommendation to improve mammary health and reproduction. The recommendation was made despite the lack of titration trials, knowing the amount of vitamin E in the basal diet would seldom be known, and realizing there could be numerous interactions with other antioxidants. Similarly, our knowledge of availability of choline from rumen-protected sources is incomplete as is our knowledge of interactions between choline and other nutrients involved with one-carbon metabolism. Nevertheless, there is overwhelming evidence that feeding transition dairy cows 15 g choline/day in a form that is protected from ruminal degradation will alleviate choline's classic deficiency symptom and lead to improvements in health and performance.

REFERENCES

- Ardalan, M., M. Dehghan-banadaky, K. Rezayazdi, and N. Ghavi Hossein-Zadeh. 2011. The effect of rumen-protected methionine and choline on plasma metabolites of Holstein dairy cows. *J. Agric. Sci.* doi:10.1017/S0021859610001292.
- Bertics, S. J., and R. R. Grummer. 1999. Effects of fat and methionine hydroxy analog on prevention and alleviation of fatty liver induced by feed restriction. *J. Dairy Sci.* 82:2731-2736.
- Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *J. Dairy Sci.* 87:3105-3124.
- Cole, L. K., J. E. Vance, and D. E. Vance. 2011. Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim. Biophys. Acta.* 1821:754-761.
- Cooke, R. F., N. Silva Del Rio, D. Z. Caraviello, S. J. Bertics, M. H. Ramos, and R. R. Grummer. 2007. Supplemental choline for prevention and alleviation of fatty liver in dairy cattle. *J. Dairy Sci.* 90: 2413-2418.
- Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in the liver of dairy cows during the periparturient period. *J. Dairy Sci.* 84(E. Suppl.):E100-112.
- Elek, P., J. R. Newbold, T. Gaal, L. Wagner, and F. Husveth. 2008. Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. *Anim.* 2:1595-1601.
- Goselink, R., J. van Baal., A. Widaja, R. Dekker, R. Zom., M. J. de Veth, and A. van Vuuren. 2012. Regulation of hepatic triacylglycerol level in dairy cattle by rumen-protected choline supplementation during the transition period. *J. Dairy Sci.* 95:submitted.
- Grummer, R. R. 1993. Etiology of lipid related metabolic disorders in periparturient dairy cattle. *J. Dairy Sci.* 76:3882-3896.
- Hartwell, J. R., M. J. Cecava, and S. S. Donkin. 2000. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. *J. Dairy Sci.* 83:2907-2917.
- Guretzky, N. A., D. B. Carlson, J. E. Garret, and J. K Drackley. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *J. Dairy Sci.* 89:188-200.
- Kleppe, B. B., A. J. Aiello, R. R. Grummer, and L. E. Armentano. 1988. Triglyceride accumulation and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. *J. Dairy Sci.* 71:1813-1822.
- Lima, F. S., M. F. Sa Filho, L. F. Greco, F. Susca, V. M. Magalhaes, and J. E. P. Santos. 2007. Effects of feeding rumen-protected choline on metabolism and lactation. *J. Dairy Sci.* 90 (Suppl. 1):174
- Lima, F.S., M.F. Sa Filho, L. F. Creco, and J. E. P. Santos. 2011. Effects of feeding rumen-protected choline on incidence of diseases and reproduction in dairy cows. *Vet J.* 193(1):140-145.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*, Seventh Revised Ed. Washington, D. C.: National Academy Press.

- Osorio, J. S., P. Ji, J. K. Drackley, and J. Loo. 2011. Effects of supplemental Smartamine or MetaSmart in moderate-energy close-up diets on peripartal liver tissue composition and blood metabolites. *J. Dairy Sci.* 94(Suppl. 1):766.
- Oelrichs, W. A., M. C. Lucy, M. S. Kerley, and J. N. Spain. 2004. Feeding soybeans and rumen-protected choline to dairy cows during the periparturient period and early lactation. 2. Effects on reproduction. *J. Dairy Sci.* 87(Suppl. 1):344.
- Piepenbrink, M. S., and T. R. Overton. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. *J. Dairy Sci.* 86:1722-1733.
- Pinotti, L., A. Baldi, I. Politis, R. Rebucci, L. Sangalli, and V. Dell'Orto. 2003. Rumen-protected choline administration to transition dairy cows: effects on milk production and vitamin E status. *J. Vet. Med. A* 50:18-21.
- Preynat A, Lapierre H, Thivierge MC, Palin MF, Cardinault N, Matte JJ, Desrochers A, Girard CL. 2010. Effects of supplementary folic acid and vitamin B(12) on hepatic metabolism of dairy cows according to methionine supply. *J Dairy Sci.* 93:2130-2142.
- Pullen, D. L., J. S. Liesman, and R. S. Emery. 1990. A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media. *J. Anim. Sci.* 68:1395-1399.
- Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beaver. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.* 86:1201-1217.
- Sharma, B. K., and R. A. Erdman. 1988. Effect of high amounts of dietary choline supplementation on duodenal flow and production responses of dairy cows. *J. Dairy Sci.* 71:2670-2676.
- Socha, M. T. 1994. Determining the methionine requirements of lactating dairy cows. PhD Thesis. Univ. of New Hampshire, Durham.
- Xu, G., J. Ye, J. Liu, and Y. Yu. 2006. Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. *Asian-Aust. J. Anim. Sci.* 19:390-395.
- Zahra, L. C., T. F. Duffield, K. E. Leslie, T. R. Overton, D. Putnam, and S. J. LeBlanc. 2006. Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient cows. *J. Dairy Sci.* 89:4808-4818.
- Zom, R. L. G, J. van Baal, R. M. A. Goselink, J. A. Bakker, M. J. de Veth, and A. M. van Vuuren. 2011. Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. *J. Dairy Sci.* 94:4016-4027.

SESSION NOTES