SUPPLEMENTAL CHOLINE FOR PREVENTION AND ALLEVIATION OF FATTY LIVER IN DAIRY CATTLE

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Fatty Liver is a metabolic disorder that can affect up to 50% of the high producing cows during the transition period, potentially compromising health, production and reproduction. Fatty liver develops when plasma nonesterified fatty acid (NEFA) concentrations are high due to depressed feed intake and altered endocrine status associated with initiation of parturition and lactation. The NEFA concentration at which triglyceride (TG) begins to accumulate in liver is not well established, but is known that the hepatic uptake of NEFA is directly associated with its concentration in blood.

Accumulation of TG in the liver (i.e. fatty liver) may reduce the rate of hepatic ureagenesis, hormone degradation, and hormone responsiveness (Strang et al., 1998a, 1998b). Prevention of fatty liver may be necessary to maintain optimal hepatic function during periparturient period. Some strategies to prevent the incidence of fatty liver are suppression of fatty acid mobilization from adipose tissues and enhancement of TG export as a constituent of very low density lipoproteins (VLDL) from the liver. The application of this last strategy is challenging because ruminants have a slow rate of hepatic VLDL secretion (Grummer, 1995) and the rate limiting step for secretion has not been identified.

Choline deficiency in rats has been shown to cause an increase in accumulation of TG in liver. As a methyl donor for biochemical reactions, choline may spare the requirement for methionine (also a methyl donor). Choline also serves as a substrate for synthesis of phosphatidylcholine (PC), a constituent of VLDL. Methionine is an amino acid that is required for synthesis of protein (a constituent of VLDL) and as a methyl donor for PC synthesis. Therefore, if flow of choline to the intestine of dairy cattle is insufficient during the periparturient period when feed intake is low, synthesis of VLDL could be limited and fatty liver could result.

The microbial population in the rumen quickly degrades dietary choline. Therefore, the only effective way to assess if ruminants are deficient is to supplement choline postruminally or feed choline in a form that is protected from ruminal degradation (Atkins et al., 1998). Early studies indicated that postruminal delivery of choline could increase milk and milk fat yield (Sharma and Erdman, 1989). These researchers speculated that the increase in fat yield may have been related to improved processing of fat through the liver, but no direct evidence was provide to support that theory. Only recently has the effects of choline on liver TG been measured directly. Hartwell et al. (2000) fed ruminally protected choline to transition dairy cows but did not see any beneficial effect on liver TG concentration. However, the degree of ruminal protection of the choline fed in that trial has been questioned by the manufacturer of the product (D. Putnam, personal communication). More recently, an improved protected choline product (D. Putnam, personal communication) was fed to transition dairy cows and a statistically non-significant reduction in liver TG was observed as level of supplementation was increased (Piepenbrink and Overton, 2004). Liver TG is a highly variable measurement in dairy cattle immediately after parturition and this study may not have had adequate animals numbers to detect statistically significant treatment differences. Therefore, we attempted to access whether choline had a role in preventing or alleviating fatty liver using an experimental model that might be more sensitive for detecting a treatment effect.

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The objective of our study was to observe if rumen protected choline (RPC) could reduce TG concentration in liver during negative energy balance when hepatic TG is accumulating and after a period of negative energy balance when positive energy balance is restored and TG is being depleted from the liver. We decided to test the ability of RPC to prevent or alleviate fatty liver using far-off dry cows that are usually much less variable than transition dairy cows. All animals were pregnant, nonlactating, multiparous Holstein cows. They were housed in individuals tie stalls bedded with sawdust.

Experiment 1: Experiment 1 was conducted to determine if RPC (Balchem Encapsulates - Slate Hill, NY) reduces hepatic TG accumulation during negative energy balance. Twenty-four cows between 45 d to 60 d prepartum were blocked by BCS and randomly assigned to one of two treatments: 1) control, or 2) 60g of RPC/day. The experimental period was 17 days. From days 0 to 6, cows were fed forage twice a day at 0730 and 1500 h. Corn silage and alfalfa silage were fed ad libitum (1:1 DM basis). Vitamins and minerals to meet requirements

were fed 30 min prior to morning forage feeding via 1.4 kg of a corn-based concentrate. On day 7, cows were the restricted to 30% of maintenance requirements for energy to induce fatty liver. During feed restriction, cows continued to receive vitamins and minerals to meet requirements via 1.4 kg concentrate per day. Blood was sampled from coccygeal vein or artery 2 h before feeding, immediately prior to morning feeding and 2, 4 and 6 h after morning feeding on days 5 and 16. Liver samples were obtained via needle biopsy on days 6 and 17. Measurements made on days 5 and 6 served as covariables for statistical analysis.

Experiment 2: Experiment 2 was conducted to determine if RPC would alter the rate of TG depletion from liver when cows are in positive energy balance following induction of fatty liver by feed restriction. Twenty-eight cows between 45 to 60 days prepartum were blocked by BCS and randomly assigned to one of two treatments: 1) control, 2) 60g of RPC/day. Two groups of fourteen cows each (7 blocks per group) went through the protocol at separate times. Prior to receiving treatments, cows were restricted to 30% of their maintenance requirements for energy for 10 days as described in experiment 1. On the last day of feed restriction, blood was sampled from the coccygeal vein or artery immediately prior to and 4 h after feeding. A liver biopsy was obtained following the last blood sample and these measurements were used as covariables. For the next 6 days, cows were allowed to consume corn and alfalfa silage (1:1 DM basis) ad libitum and 1.4 kg/day of concentrate containing vitamins and minerals to meet requirements and initiate depletion of TG from the liver. During this time 0 or 60 g/d RPC were fed. Cows were fed forage twice a day (0730 and 1500 h) and the concentrate was offered 30 minutes before morning feeding. Dry matter intake was measured daily during this period. Blood (immediately prior to and 4 h after morning feeding) and liver (after 4 h blood sample) samples were obtained on day 3 and 6 following feed restriction.

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Experiment 1: Plasma concentrations of glucose, NEFA, and β–hydroxybutyrate (BHBA) during feed restriction and treatment are in Table 1. No time by treatment interactions were detected, so data is pooled across all sampling times. RPC supplementation had a tendency to reduce plasma glucose ($P < 0.15$). Since feed intake was restricted during the sampling period, differences in plasma glucose are independent of energy intake. Plasma NEFA concentration was reduced during RPC supplementation ($P < 0.005$). Lower plasma NEFA concentration was not mirrored by a similar reduction in BHBA.

Liver TG prior to feed restriction was 1.0 and 1.1 μg TG/μg DNA in cows destined to receive concentrate without or with supplemental RPC. Feeding RPC resulted in a significant decrease ($P < 0.02$) in the extent of TG accumulation during feed restriction (Figure 1).

Experiment 2: Dry matter intake following feed restriction and during positive energy balance was slightly lower for cows fed supplemental RPC but the difference between treatments was not statistically significant ($P < 0.28$) (Figure 2).

Plasma glucose, NEFA, and BHBA concentrations in cows fed ad libitum following feed restriction were not affected by RPC supplementation (Table 2). Glucose concentrations were higher and NEFA and BHBA concentrations were lower than during experiment 1 which would be expected based on greater energy intake.

Liver TG values for cows destined to receive control or RPC supplemented concentrate during ad libitum feed consumption following feed restriction were 6.7 and 12.7 μg/μg DNA, respectively. This was not anticipated since no treatment had begun and cows were randomly assigned to treatment. This outcome, although not expected, happened by chance. Pretreatment liver TG concentration was a highly significant covariate ($P < 0.001$). That indicates that there was a relationship between pretreatment liver TG and liver TG during treatment. Consequently, it is critically important to examine day 3 and day 6 data as covariately adjusted means. Doing so indicated that RPC supplementation increased the rate of TG depletion from the liver during positive energy balance. Liver TG at 3 and 6 d as a percentage of TG pretreatment was 60.4 and 48.5 for cows on control and 52.2 and 29.9 for cows fed RPC supplemented concentrate ($P < 0.12$ for treatment, $P < 0.07$ for time × treatment; SE = 6).

Discussion

These experiments were designed to determine if supplemental RPC could prevent or alleviate fatty liver that was caused by elevated NEFA concentrations during feed restriction. We avoided the use of transition cows in a randomized design because of the tremendous amount of animal variation during the periparturient period and the large number of animals that are required to adequately test for treatment effects on liver TG. Instead, we used feed restriction to mimic the decline in feed intake that typically occurs near the time of

parturition and we used far off cows because our experience indicates that they are a relatively homogeneous group of cows.

Results from the first study indicate that supplemental RPC can reduce TG accumulation in the liver during negative energy balance. The mechanism by which RPC reduces liver TG is unknown. Choline deficiency in laboratory animals can result in fatty liver. Presumably, under such conditions, phosphatidylcholine synthesis is limited which in turn limits TG export out of the liver as a constituent of VLDL. However, in our study, plasma NEFA were reduced through RPC supplementation. Since NEFA uptake by the liver is a function of blood flow to the liver and NEFA concentration in blood, it is possible that some or all of the reduction in liver TG may have been due to an indirect effect of choline reducing plasma NEFA vs. a direct effect on enhancing TG export from the liver.

The results of experiment 2 are complimentary to those from experiment 1. They also suggest that supplemental choline may enhance VLDL export from the liver and serve a role in the alleviation of fatty liver. Treatment effects were observed without changes in plasma NEFA suggesting a direct effect of choline on the liver. However, these results must be interpreted with caution because of the discrepancy in liver TG in the two groups prior to treatment. Raw means for liver TG (μg/μg DNA) in cows on control and RPC at day 3 following feed restriction were 3.8 and 7.5 and at day 6 they were 3.2 and 4.1. Comparison of these numbers with the covariately-adjusted means illustrates the huge impact of covariate analysis. Consequently, the amount of faith one has in these data is proportional to the amount of confidence one has in covariate analysis to correct for the discrepancies in liver TG observed pretreatment.

Implications

Rumen protected choline supplementation of dairy diets may lead to a reduction in fatty liver during times of negative energy balance. Most strategies to prevent fatty liver have depended on nutritional supplements to reduce fat mobilization from body stores (e.g. propylene glycol or niacin). In contrast, choline may act directly on the liver to increase export of fat. This is the preferential strategy to prevent fatty liver since it does not reduce or block a beneficial biological function, i.e., mobilization of energy to support lactation and function of other tissues.

Literature Cited

Atkins, K.B., R. A. Erdman, and J. H. Vandersall. 1998. Dietary Choline effects on milk yield and duodenal choline flow in dairy cattle. J. Dairy Sci. 71:109-116.

Grummer R. R. 1995. Impact of changes in organic nutrient metabolism on developing feeding strategies to minimize stress during the transition from late pregnancy to early lactation. J. Anim. Sci. 73:2820-2833.

Hartwell, J. R., M. J. Cecava, and S. S. Donkin. 2000. Impact of dietary rumen undegradable protein and choline on intake, peripartum liver triglyceride, plasma metabolites and milk production in dairy cows. J. Dairy Sci. 83:2907-2917.

Piepenbrink M.S., and T. R. Overton. 2004. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86:1722-1733.

Sharma, B. K., and R. A. Erdman.1989. Effect of dietary and abomasally infused choline on milk production responses of lactating dairy cows. J. Nutr. 119:248.

Strang, B. D., S. J. Bertics, R. R. Grummer, and L. E. Armentano. 1998a. Effect of long-chain fatty acids on triglyceride accumulation, gluconeogenesis and ureagenesis in bovine hepatocytes. J. Dairy Sci. 81:728-739.

Strang, B. D., S. J. Bertics, R. R. Grummer, and L. E. Armentano. 1998b. Relationship of triglyceride accumulation to insulin clearance and hormonal responsiveness in bovine hepatocytes. J. Dairy Sci. 81:740-747.

Table 1. Covariately-adjusted least squares means for plasma metabolites, experiment 1

	Control		RPC		*P* <
	Mean	SE	Mean	SE	
Glucose, mg/dl	48.3	1.5	51.4	1.4	0.15
NEFA, µEq/L	703	40	562	39	0.004
BHBA, mg/dl	7.6	0.3	8.0	0.4	0.48

Table 2. Covariately-adjusted least squares means for plasma metabolites, experiment 2

	Control		RPC		*P* <
	Mean	SE	Mean	SE	
Glucose, mg/dl	60.8	0.9	60.1	0.9	0.58
NEFA, µEq/L	119	6	129	6	0.31
BHBA, mg/dl	6.6	0.3	6.1	0.4	0.24

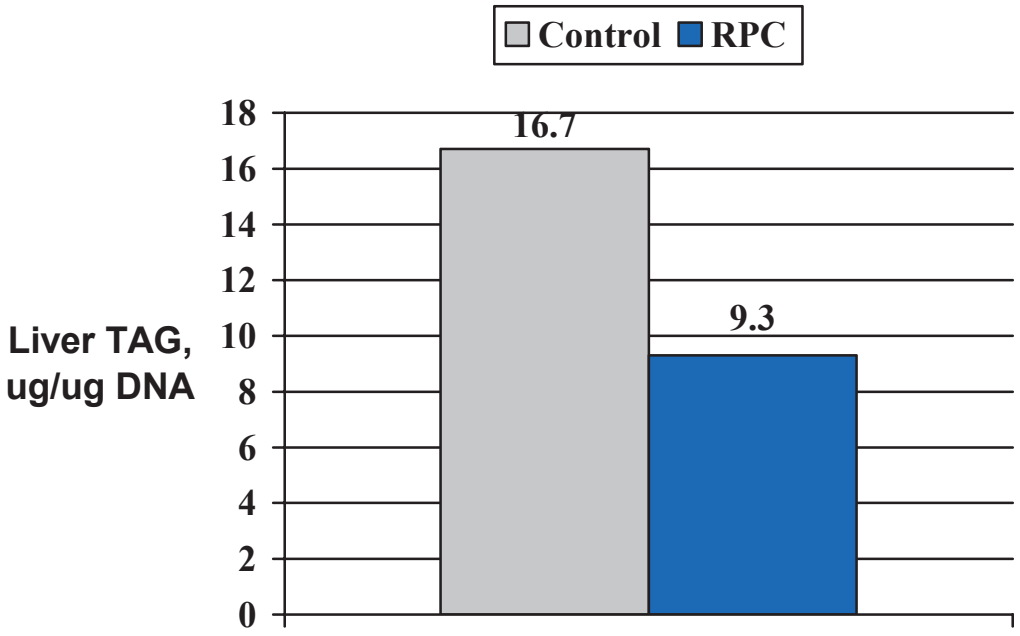

Figure 1. Covariately-adjusted least squares means for liver triglyceride (TG) in cows that were feed restricted for 10 days and fed 0 or 60 g rumen protected choline (RPC)/d. Treatment means were significantly different ($P < 0.02$). Standard errors for control and RPC were 1.80 and 2.0

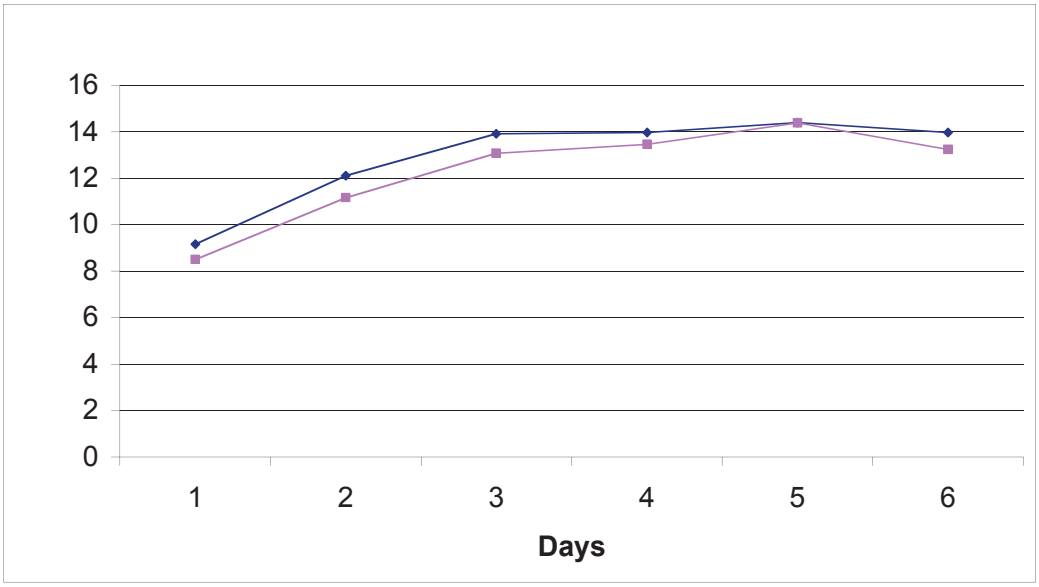

Figure 2. Covariately-adjusted least squares means for forage dry matter intake (DMI) of cows that were feed restricted to induce fatty liver and then allowed to consume feed ad libitum that was supplemented with 0 or 60 g rumen protected choline (RPC)/d. Treatment differences were not significant ($P < 0.28$). Standard error for control and RPC were 1.3 kg/d

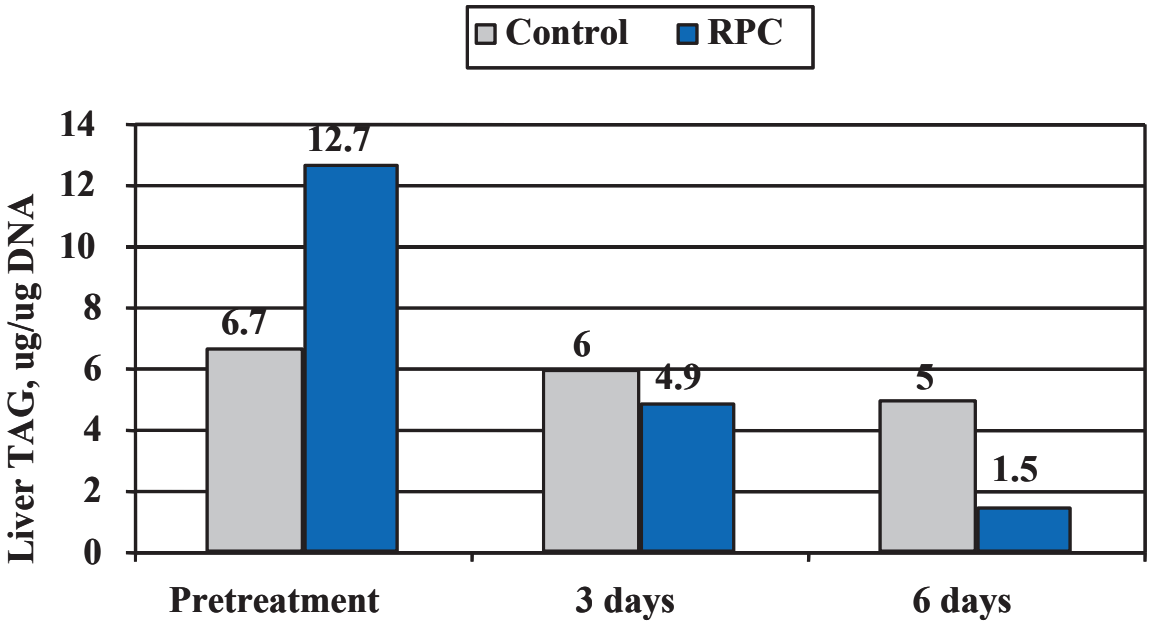

Figure 3. Liver triglyceride (TG) concentration in cows that were feed restricted to induce fatty liver (pretreatment) and at three and six days after cows were allowed to consume feed ad libitum that was supplemented with 0 or 60 g rumen protected choline (RPC)/d. Least squares means at three and six days are covariately-adjusted to account for discrepancies in pretreatment liver TG. Treatment × time, $P < 0.05$; Choline, $P < 0.02$ (SE = 0.4 at 3 days and 0.9 at 6 days)