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Feeding increasing amounts of ruminally protected choline decreased fatty liver in nonlactating, pregnant Holstein cows in negative energy status

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ABSTRACT

The objectives were to determine the optimal feeding amount of choline in a ruminally protected form to reduce the triacylglycerol (TAG) concentration in liver and to increase TAG in blood plasma of dairy cows. Pregnant, nonlactating multiparous Holstein cows (n = 77) were blocked by body condition score (3.59 ± 0.33) and assigned to treatment at 64 ± 10 d before calculated calving date. Dietary treatments were top-dressing of 0, 30, 60, 90, or 120 g/d of ruminally protected choline (RPC; Balchem Corp., New Hampton, NY) ions to supply the equivalent of 0, 6.5, 12.9, 19.4, and 25.8 g/d of choline ions. Diets were formulated to exceed nutrient requirements for maintenance and pregnancy and fed in ad libitum amounts for the first 5 d. From d 6 to 15, cows were restricted to consume approximately 31% of their net energy requirements to simulate early lactating cows in negative energy balance. Methionine intake was maintained throughout each 15-d period. Liver was biopsied at 5 and 14 d and analyzed for TAG and glycogen. Blood was sampled on d 5 and 14 and plasma analyzed for glucose, insulin, cholesterol, β-hydroxybutyrate, long-chain fatty acids, and haptoglobin. On d 14, a mixture of saturated long-chain fatty acids, ground corn, and dried molasses (50:37:13) was offered (908 g, as-is basis) 10 h after the single daily feeding. Blood samples were collected for 19 h and plasma analyzed for TAG and cholesterol to assess apparent absorption of dietary fat. Mean dry matter intake and energy balance decreased from means of 9.5 to 3.3 kg/d and from 0.6 to −9.2 Mcal of net energy for lactation/d during the ad libitum and restricted feeding periods, respectively. Plasma concentrations of the lipid-soluble choline biomolecules, namely total phosphatidylcholines, total lysophosphatidylcholines, and sphingomyelin, increased with choline supplementation. Feed restriction increased plasma concentrations of β-hydroxybutyrate and free long-chain fatty acids, whereas those of glucose, insulin, and total cholesterol decreased. During feed restriction, concentration of hepatic TAG and plasma haptoglobin decreased linearly, whereas concentration of hepatic glycogen tended to increase quadratically with increasing intake of RPC. After fat supplementation, mean plasma concentration of TAG increased by an average of 21% with intake of RPC ions, peaking at intakes of ≥6.5 g/d of RPC ion. In summary, feeding RPC ions to cows in negative energy balance had increasing lipotropic effects on the liver when consumed up to 25.8 g/d, whereas feeding only 6.5 g/d increased concentrations of hepatic glycogen and TAG in the blood.
Key words: choline, fatty liver, triacylglycerol

INTRODUCTION

Usually the liver accumulates triacylglycerol (**TAG**) during the periparturient period in dairy cows (Bobe et al., 2004) as energy balance (**EB**) becomes negative. High concentrations of circulating long-chain fatty acids from mobilized TAG from adipose tissue are the precursors for the accumulation of TAG in the liver. Extensive re-esterification of long-chain fatty acids in hepatocytes and the low rates of export of TAG as a key structural component of very low density lipoproteins (**VLDL**) have been suggested as the cause for development of fatty liver (Grummer, 1993). Ruminant liver has an inherently very low capability of secreting VLDL (Kleppe et al., 1988).

Choline, a micronutrient often classified with the B-vitamins, is a proven lipotropic agent in several species of animals. The daily adequate intake of choline established for adult humans was based on the amount of choline consumed to prevent liver dysfunction (Jiang et al., 2014). Steatosis was observed within 1 wk

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of withdrawing choline from the diet of rats, but the combination of choline and methionine deficiency was more damaging to the liver (Vetelainen et al., 2007). The quantification of VLDL as a limiting transporting molecule to reduce TAG accumulation in cows is lacking. However, treating primary hepatocytes from neonatal Holstein calves with increasing concentrations of choline chloride (0, 61, 128, 2,028, and 4,528 μmol/L) increased the secretion of VLDL in the medium (Chandler and White, 2017). The supplementation of ruminally protected choline (**RPC**) ions to periparturient dairy cows (from approximately 21 d before calving through at least 21 d postpartum) decreased the postpartum concentration of hepatic TAG in some (Santos and Lima, 2009; Zom et al., 2011; Elek et al., 2013) but not in other experiments (Zahra et al., 2006; Zhou et al., 2016b; Zenobi et al., 2018). The source (ReaShure, Balchem Corp., New Hampton, NY) and amount of RPC ions fed (approximately 12.9 g/d) were the same across these studies with the exception of Elek et al. (2013) who fed 18.6 g/d prepartum and 37.3 g/d postpartum of RPC ions from a different commercial source (Norcol-25, Nordos, Bussolengo, Italy). Only Piepenbrink and Overton (2003) fed multiple amounts of RPC ions in the same study. They reported that hepatic concentration of TAG decreased numerically on 1 and 21 DIM with increasing intake of RPC ions (0, 9.7, 12.9, and 16.1 g/d) but the trend was not significant (linear effect, $P = 0.18$). However, the in vitro conversion of carbon-14 palmitate to esterified products in biopsied liver tissue tended to decrease ($P = 0.06$) linearly by 18% with increasing intake of RPC ions. In addition, the hepatic concentration of glycogen increased linearly with increasing intake of RPC ions.

Supplemental choline may improve the performance of lactating dairy cows through additional mechanisms such as improved intestinal function. The intestinal brush border membrane in rats is composed of equal proportions of phosphatidylcholine (**PC**) and its precursor, phosphatidylethanolamine (**PE**), totaling 64% of the phospholipids (Christon et al., 1991). In addition, PC is an integral part of the chylomicron molecule, which contains 20% phospholipid (Hartmann and Lascelles, 1966) of which 100% was PC (Jenkins et al., 1988) in bovine. Chylomicrons are critical to the movement of lipids and fat-soluble vitamins from the small intestine to the lymphatic vessels for transfer to the blood (Bauchart, 1993). Rats fed a choline-deficient diet and given corn oil by gavage accumulated lipid droplets within the absorptive cells of the small intestine and had a decreased proportion of lipoproteins as chylomicrons in intestinal lymph compared with rats fed a choline-adequate diet. This was corrected when rats received PC by gavage (Takahashi et al., 1982a). Secretion of chylomicrons was impaired and intestinal villi were shorter when lactating rats were fed a diet deficient in choline (da Silva et al., 2015). Intestinal integrity and plasma concentration of TAG was improved when a choline deficiency was corrected in lactating rats (da Silva et al., 2015). The protective barrier of the small intestine in transition dairy cows deteriorated with decreased feed intake and was accompanied by a linear increase in plasma concentration of haptoglobin (Kvidera et al., 2017). Supplemental RPC ions may have beneficial effects during this time period.

The objectives of the current study were to determine the optimal feeding amount of RPC ions to reduce the concentration of TAG in liver and to increase the concentration of TAG in blood of pregnant multiparous Holstein cows in a negative energy state. Based on the recommended adequate intake of choline for a pregnant woman of 450 mg/d (Jiang et al., 2014) and the ruminal escape of RPC (ReaShure) of 86.1% (Elek and Husvéth, 2007), our hypothesis was that supplementing 12.9 to 19.4 g/d of choline ions to pregnant multiparous Holstein cows in negative EB would optimize concentrations of hepatic and plasma TAG. This animal model was chosen over lactating dairy cows to minimize the variation in EB that exists among newly lactating dairy cows compared with nonlactating dairy cows and still use an animal model that responds to supplemental RPC ions (Cooke et al., 2007). The liver of nonlactating and lactating dairy cows responded similarly to feed restriction (Reid et al., 1979).

MATERIALS AND METHODS

Cows

The experiment was conducted at the University of Florida dairy farm from December 2015 to June 2016. Mean and range of daily temperatures were 17.4°C and 2.0 to 28.7°C and those of daily relative humidity were 75.1% and 46 to 97%, respectively. All procedures involving cows in the experiment were carried out according to the University of Florida's Institutional Animal Care and Use Committee. Seventy-seven pregnant, nonlactating multiparous Holstein cows were enrolled in the experiment at 64 ± 10 d (average ± SD; range of 41 to 88 d) before the calculated calving date. The mean ± standard deviation and range at enrollment were 3.59 ± 0.33 and 2.75 to 4.25 for BCS and 732 ± 96 kg and 530 to 944 kg for BW, respectively. Mean number and range of lactations was 1.9 and 1 to 5. Cows were dried off at the beginning of the experiment and moved to indoor tiestalls each containing an indi-