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Relative bioavailability of carnitine delivered by ruminal or abomasal infusion or by encapsulation in dairy cattle

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ABSTRACT

Two studies were designed to evaluate the relative bioavailability of L-carnitine delivered by different methods in dairy cattle. In experiment 1, 4 Holstein heifers were used in a split-plot design to compare ruminally or abomasally infused L-carnitine. The study included 2 main-plot periods, with infusion routes allocated in a crossover design. Within main-plot periods, each of 3 subplot periods consisted of 4-d infusions separated with 4-d rest periods. Subplot treatments were infusion of 1, 3, and 6 g of L-carnitine/d in conjunction with 6 g/d of arabinogalactan given in consideration of eventual product manufacturing. Doses increased within a period to minimize carryover risk. Treatments were solubilized in 4 L of water and delivered in two 10-h infusions daily. Blood was collected before the start of infusion period and on d 4 of each infusion period to obtain baseline and treatment L-carnitine concentrations. There was a dose × route interaction and route effect for increases in plasma carnitine above baseline, with increases above baseline being greater across all dose levels when infused abomasally compared with ruminally. Results demonstrated superior relative bioavailability of L-carnitine when ruminal exposure was physically bypassed. In experiment 2, 56 lactating Holstein cows (143 ± 72 d in milk) were used in 2 cohorts in randomized complete block designs (blocked by parity and milk production) to evaluate 2 rumen-protected products compared with crystalline L-carnitine. Treatments were (1) control, (2) 3 g/d of crystalline L-carnitine (crystalline), (3) 6 g/d of crystalline, (4) 5 g/d of 40COAT (40% coating, 60% L-carnitine), (5) 10 g/d of 40COAT, (6) 7.5 g/d of 60COAT (60% coating, 40% L-carnitine), and (7) 15 g/d of 60COAT. Treatments were top-dressed to diets twice daily. Each cohort used 14-d and included a 6-d baseline measurement period with the final 2 d used for data and sample collection, and an 8-d treatment period with the final 2 d used for data and sample collection. Plasma, urine, and milk samples were analyzed for L-carnitine. Crystalline and 40COAT linearly increased plasma L-carnitine, and 60COAT tended to linearly increase plasma L-carnitine. Total excretion (milk + urine) of L-carnitine averaged 1.52 ± 0.04 g/d in controls, increased linearly with crystalline and 40COAT, and increased quadratically with 60COAT. Crystalline increased plasma L-carnitine and L-carnitine excretion more than 40COAT and 60COAT. In conclusion, preventing ruminal degradation of L-carnitine increased delivery of bioavailable carnitine to cattle, but effective ruminal protection and postruminal bioavailability is challenging.
Key words: L-carnitine, bioavailability, dairy cow

INTRODUCTION

Fatty liver is a metabolic disease that occurs during the first few weeks of lactation and affects up to 50% of dairy cows (Grummer, 1993; Jorritsma et al., 2000). Dry matter intake declines around calving, resulting in rapid lipolysis of adipose tissue triacylglycerides (**TAG**) to provide an insulin-independent energy source for tissues and to support gluconeogenesis. Hepatic uptake of fatty acids often occurs in excess of its oxidation capacity with resultant re-esterification and storage of TAG (Vernon, 2005). Liver lipid accumulation damages hepatocytes, decreasing metabolic function of the liver, and fatty liver has been associated with decreased health status and reproductive performance (Wensing et al., 1997; Bobe et al., 2004). Key control points for hepatic lipid metabolism include delivery of fatty acids to the liver and transport into the mitochondria by carnitine palmitoyltransferase 1 (Drackley, 1999).

L-Carnitine plays an essential role in the transport of long-chain fatty acids (**LCFA**) from the cytosol into the mitochondria of hepatocytes (Longo et al., 2006). The conjugation of L-carnitine with LCFA by CPT1 in the outer mitochondrial membrane is considered the

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rate-limiting step in carnitine-dependent fatty acid oxidation (Louet et al., 2001). From 3 wk before calving to 1 wk postpartum, the concentration of free carnitine decreased while acylcarnitine concentration increased; however, total postpartum carnitine concentration (free carnitine + carnitine esters) was decreased relative to prepartum values (Schlegel et al., 2012). Cows that rapidly mobilize body fat, grouped by postpartum liver lipid content, exhibited elevated concentrations of acylcarnitines and decreased free carnitine concentrations (Humer et al., 2016). Hepatic mRNA abundance of CPT1 was greatest around d 1 of lactation, coinciding with peak levels of plasma fatty acids (Ingvartsen and Anderson, 2000; Loor et al., 2005; Schlegel et al., 2012). Increased CPT1 mRNA expression was also observed in cows with greater BCS at calving (Akbar et al., 2015). Increased mRNA abundance of carnitine palmitoyltransferase 1 and decreased free hepatic carnitine concentrations suggest that exogenous carnitine supply could enhance transport and oxidation of LCFA in the postpartum period, particularly in cows with the greatest elevations in plasma fatty acid concentration.

In vitro incubation of liver slices from early lactation cows with L-carnitine increased β-oxidation of palmitate and decreased palmitate esterification (Drackley et al., 1991). Dietary administration of L-carnitine during the transition period was effective at increasing hepatic carnitine concentration, with a subsequent decrease in total liver lipid content at 10 DIM, the time of peak liver TAG concentration (Drackley et al., 2005; Carlson et al., 2007a).

Carnitine is degraded in the rumen, but the extent of degradation is unknown. Abomasal and ruminal infusions of carnitine (6 g/d) were equally effective at increasing plasma carnitine concentrations in one study, suggesting some carnitine is able to escape ruminal degradation and be available for intestinal absorption (LaCount et al., 1995). Previous studies have assumed 80% ruminal degradation for lactating dairy cows fed a commercial carnitine supplement (6, 50, and 100 g/d of a 50% L-carnitine product; Carlson et al., 2007a), and 25% absorption was speculated for growing steers supplemented at 2 and 3 g of carnitine per day (Greenwood et al., 2001).

More research is needed to determine the extent of ruminal carnitine degradation to evaluate the necessity for a rumen-protected form of L-carnitine. The objective of experiment 1 was to assess the relative bioavailability of L-carnitine when administered at different sites and different infusion rates, whereas experiment 2 assessed the relative bioavailability of 2 rumen-protected carnitine (**RPC**) products compared with crystalline carnitine. Experiment 2 also determined production responses to supplemental carnitine, including milk yield, milk components, and feed intake. We hypothesized that ruminal microbial degradation would reduce relative bioavailability of ruminally administered carnitine and that feeding rumen-protected products would result in greater relative carnitine bioavailability than crystalline carnitine. Relative change in plasma carnitine concentration was used to assess relative bioavailability.

MATERIALS AND METHODS

Experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (protocols 3300 and 3653).

Experiment 1

Four Holstein heifers previously fitted with ruminal cannulas were used in a split-plot design to assess the relative bioavailability of ruminally or abomasally administered L-carnitine. However, 1 heifer was removed just before the end of the first treatment period due to an intestinal blockage requiring surgery. A second heifer was removed due to an infection during phase 2 of period 2. At that time, the heifer removed in period 1 had recovered and was used to replace the second heifer for phase 3 of period 2. The study was therefore an incomplete design. Heifers were housed in a tiestall facility and fed a TMR once daily (Table 1). The diet met requirements for all nutrients (NRC, 2001).

The study was conducted in 2 periods, both preceded by 2 wk without treatment to obtain baseline samples and for washout between periods. Each period had 3 phases, each consisting of 4 d of infusions at a different dose of carnitine, with 4 d between phases. The treatments included ruminal infusion of 1, 3, and 6 g of L-carnitine/d and abomasal infusion of 1, 3, and 6 g of L-carnitine/d. Each carnitine treatment also included 6 g/d of larch arabinogalactan in consideration of eventual product manufacturing, and was solubilized in water (pH = 9.4) with the total volume infused being 4 L/d across treatments. The dosage used in each phase escalated, with phase 1 at 1 g/d, phase 2 at 3 g/d, and phase 3 at 6 g/d, to minimize the relative effect of any carryover. Doses were based on the previous dose response study by LaCount et al. (1996a) in which plasma carnitine concentration appeared to be maximized at 6 g L-carnitine/d. The site of infusion was randomized; 2 heifers received ruminal infusions in period 1, followed by abomasal infusions in period 2, and the other heifer was treated in the opposite sequence. Daily infusions (throughout each 4-d infusion) were split into 2 equal aliquots, each infused during 10-h infusion periods, allowing 2 h between infusions.