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K. Olagaray

Kansas State University, Manhattan, kolagaray@k-state.edu

C. Armendariz

Kansas State University, Manhattan, cheryl@ksu.edu

A. Bellamine

Lonza, Inc., Allendale, NJ

See next page for additional authors

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Bioavailability of Ruminally or Abomasally Infused L-carnitine in Holstein Heifers

Abstract

This study evaluated the relative bioavailability of carnitine delivered by different methods in dairy cattle. Four 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 L-carnitine daily. Doses were increased within a period to minimize carryover. Treatments were delivered in two 10-h infusions daily. Blood was collected before the start of infusions and on day 4 of each infusion to obtain baseline and treatment carnitine concentrations. There was a dose \times route interaction ($P < 0.05$) and route effect ($P < 0.01$) for increases in plasma carnitine above baseline, with increases above baseline being greater across all dose levels when infused abomasally compared to ruminally. Results demonstrated superior bioavailability of carnitine when ruminal exposure was physically bypassed.

Keywords

L-carnitine, bioavailability, dairy cow

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Authors

K. Olagay, C. Armendariz, A. Bellamine, S. Jacobs, E. Titgemeyer, and B. Bradford

Bioavailability of Ruminally or Abomasally Infused L-carnitine in Holstein Heifers

K. Olgaray, C. Armendariz, A. Bellamine,¹ S. Jacobs,¹ E. Titgemeyer, and B.J. Bradford

Summary

This study evaluated the relative bioavailability of carnitine delivered by different methods in dairy cattle. Four 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 L-carnitine daily. Doses were increased within a period to minimize carryover. Treatments were delivered in two 10-h infusions daily. Blood was collected before the start of infusions and on day 4 of each infusion to obtain baseline and treatment carnitine concentrations. There was a dose \times route interaction ($P < 0.05$) and route effect ($P < 0.01$) for increases in plasma carnitine above baseline, with increases above baseline being greater across all dose levels when infused abomasally compared to ruminally. Results demonstrated superior bioavailability of carnitine when ruminal exposure was physically bypassed.

Key words: L-carnitine, bioavailability, dairy cow

Introduction

Fatty liver is a metabolic disease that commonly affects postpartum dairy cows. In response to negative energy balance that typically occurs in early lactation when feed intake is insufficient to meet the high energy demand of lactation, fatty acids are released from adipose tissue stores as an energy source. However, this lipid mobilization can deliver fatty acids to the liver at a rate that exceeds the organ's oxidative capacity, resulting in accumulation of liver lipids which is associated with decreased metabolic function. L-carnitine plays an essential role in the transport of long chain fatty acids from the cytosol into the mitochondria of hepatocytes. Increased transport of these fatty acids can potentially stimulate hepatic long chain fatty acid oxidation, thereby limiting lipid accumulation.

It has been clearly demonstrated that carnitine can be degraded by ruminal microbes, but the extent of ruminal degradation is unknown. Abomasal and ruminal infusions of carnitine have previously been equally effective at increasing plasma carnitine concentrations, suggesting some carnitine might escape ruminal degradation and be available

¹ Lonza, Inc., Allendale, NJ.

for intestinal absorption. It has been suggested that degradation rate may be dependent on diet composition and the length of time animals are fed supplemental carnitine, as ruminal microbes seem to adapt to carnitine supplementation by increasing degradation rate. Previous studies have assumed up to 80% of supplemental carnitine is ruminally degraded in lactating dairy cows. The objective of this study was to evaluate the relative bioavailability of carnitine when administered at different sites in the rumen gastrointestinal tract at varying rates.

Experimental Procedures

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, one heifer was removed just prior to 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, and the first heifer removed from the study replaced her at that time. The study was therefore an incomplete design. Heifers were housed in a tie-stall facility and fed a dairy ration once daily. The diet met estimated requirements for all nutrients and was supplemented with niacin (7.8 g/day niacin in the form of 12 g/day Niashure, Balchem Corp., New Hampton, NY).

The study was conducted in 2 periods, both preceded by 2 weeks without treatment to obtain baseline samples and for washout between periods. Each period had 3 phases, each consisting of 4 days of infusions at a different dose of carnitine, with 4 days between phases. The treatments were 1) ruminal infusion of carnitine at 1, 3, and 6 g carnitine/day and 2) abomasal infusion of 1, 3, and 6 g carnitine/day. Each carnitine treatment was dissolved in water and also included 6 g/day of larch arabinogalactan, and total volume infused was 4 L/day across treatments. The dosage used in each phase escalated, with phase 1 at 1 g/day, phase 2 at 3 g/day, and phase 3 at 6 g/day. 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-day infusion) were split into 2 equal aliquots, each infused during 10-hour infusion periods, allowing 2 hours between infusions.

Throughout the study, feed and water intake were recorded daily with the final three days of each infusion phase used for analysis. Total mixed ration samples were collected every two weeks and composited for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY; Table 1). Health was monitored daily.

Prior to the start of infusions and at 1.5 hours after initiation of the first daily infusion on day 4 of each phase, blood samples (coccygeal vein) were collected to obtain baseline and treatment carnitine concentrations. Concentrations of total carnitine in plasma were determined by an enzymatic radioisotope method.

Statistical analysis was performed using JMP (version 12, SAS Institute, Cary, NC). Dependent variables (feed intake, water intake, and change in plasma carnitine concentration) were analyzed to determine the fixed effects of route of administration, dose of carnitine, and their interaction along with the random effects of heifer and phase within period. Contrast statements were used to statistically test linear regression coef-

ficients with increasing doses for ruminal vs. abomasal infusions, and least square means were regressed against dose for the 2 infusion routes to assess relative bioavailability. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P \leq 0.10$.

Results and Discussion

Water intake was not affected by carnitine infusion across dose or route (all $P > 0.40$; Table 2). Although not affected by infusion route ($P = 0.13$), dry matter intake (DMI) did tend to increase quadratically with carnitine dose ($P = 0.07$), being highest for the 3 g/day carnitine. The tendency for a DMI effect is likely the result of our small sample size and was largely driven by data from one heifer. Previous studies have not documented DMI responses when carnitine was infused abomasally or ruminally up to 12 g/d. When carnitine was abomasally infused at a high rate (100 g/d), DMI was decreased during the first two weeks of lactation.

Plasma carnitine concentrations are reported as the difference between baseline and treatment concentrations in Table 2. A dose \times route interaction was observed ($P = 0.045$), which can largely be attributed to the linear increase in plasma carnitine concentrations with increased dose for abomasal infusion, without a significant effect for ruminal infusions. A route response was observed ($P = 0.005$) with carnitine being more bioavailable across all dose levels when infused abomasally compared to ruminally. Interestingly, increases in plasma carnitine concentrations in response to ruminal infusion appeared to plateau at 3 g/d; this could be impacted by the sequence of treatments, given that adaptation of ruminal microbes may enhance carnitine degradation after a longer period of exposure. It is also possible that L-carnitine transport from the gut reaches an upper limit at these doses. To further characterize the relative bioavailability of carnitine via these 2 routes of administration, a dose-response analysis was conducted (Figure 1). This assessment suggests that the relative bioavailability of carnitine is greater when supplied to the abomasum vs. the rumen. It should be noted that this assumes that increases in plasma concentration are directly related to the amount of carnitine absorbed.

Conclusion

Carnitine is likely degraded in the rumen, and although the extent of degradation remains unknown, our findings clearly indicate abomasal administration of carnitine results in superior bioavailability. Dietary supplementation with rumen encapsulation may be most effective to maximize carnitine delivery and absorption in the small intestine.

Table 1. Ingredient and nutritional composition of the basal diet

Item	Value
Ingredient, % of dry matter	
Alfalfa hay	21.0
Grass hay	1.7
Corn silage	16.1
Wet corn gluten feed ¹	25.7
Cotton seed	4.4
Fine rolled corn	20.4
Micronutrient premix ²	10.7
Nutrient, % of dry matter (unless otherwise specified)	
Dry matter, % as-fed	53.5
Crude protein	17.9
Acid detergent fiber	24.75
Neutral detergent fiber	43.8
Lignin	4.55
Non-fiber carbohydrate	26.75
Starch	17.9
Crude fat	4.75
Net energy for lactation, ³ Mcal/lb	0.73

¹Sweet Bran (Cargill Inc., Blair, NE).

²Premix consisted of 58.8% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 11.8% limestone, 1.47% stock salt, 1.47% trace mineral salt, 1.47% potassium chloride, 10.3% sodium bicarbonate, 2.35% magnesium oxide, 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.12% Zinpro 100 (Zinpro Corp.), 0.25% selenium premix (0.06%), 0.15% vitamin A premix (30 kIU/g), 0.04% vitamin D premix (30 kIU/g), 1.47% vitamin E premix (48 kIU/g), 0.01% ethylenediamine dihydriodide premix, 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 1.84% XP Yeast (Diamond V, Cedar Rapids, IA), 0.92% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), and 7.35% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ).

Table 2: Effect of carnitine infusion on intake performance and plasma carnitine concentration

Item	Ruminal infusion (/day)			Abomasal infusion (/day)			pSEM ¹	P - value		
	1 g	3 g	6 g	1 g	3 g	6 g		Dose	Route	Dose × route
DMI, kg/d	18.01	18.76	18.87	16.84	19.01	17.43	0.97	0.07	0.13	0.35
Water intake, L/d	7.71	8.04	8.60	8.27	9.24	7.88	0.69	0.66	0.58	0.41
Plasma, ² μM	-0.57	12.33	9.04	4.54	20.47	35.90	4.82	0.099	< 0.01	0.045

¹Reported SEM is pooled across route and dose levels.

²Plasma concentrations reported are the difference between baseline and treatment concentrations.

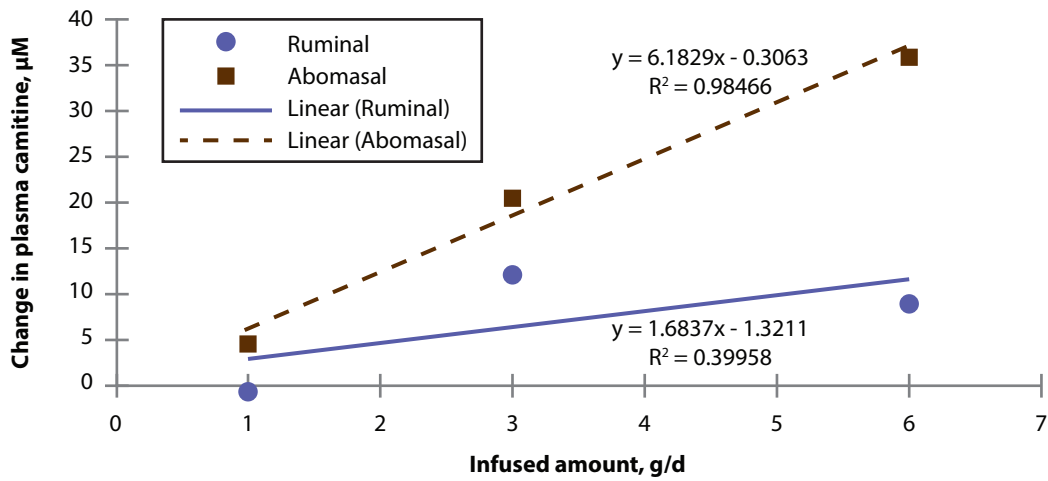


Figure 1. Marginal plasma carnitine responses to carnitine infusion differ by infusion route. Differences in plasma carnitine concentrations (post minus pre-infusion concentrations) are plotted against infusion amount. The slopes differ between infusion routes ($P = 0.02$), reflecting greater apparent bioavailability for abomasally-delivered carnitine compared to ruminal infusion.