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# Bioavailability of Rumen-Protected Carnitine in Lactating Dairy Cows

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## Summary

For this study, 56 lactating Holstein cows (143 ± 72 days in milk) were used in a randomized complete block design to evaluate 2 rumen-protected products compared to crystalline carnitine. Treatments were a) control, b) 3 grams/day crystalline L-carnitine (raw), c) 6 grams/day raw, d) 5 grams/day 40COAT (40% coating, 60% L-carnitine), e) 10 grams/day 40COAT, f) 7.5 grams/day 60COAT (60% coating, 40% L-carnitine), and g) 15 grams/day 60COAT. Treatments were top-dressed to diets twice daily. The 14-day experiment included a 6-day baseline-measurement period with the final 2 days used for data and sample collection and an 8-day treatment period with the final 2 days used for data and sample collection. Plasma, urine, and milk samples were analyzed for L-carnitine. Crystalline ($P < 0.001$) and 40COAT ($P = 0.01$) linearly increased plasma L-carnitine, and 60COAT tended to linearly increase plasma L-carnitine ($P = 0.08$). Total daily excretion (milk + urine) of L-carnitine averaged 1.52 ± 0.04 grams in controls, increased linearly with crystalline and 40COAT, and increased quadratically with 60COAT (all $P < 0.05$). Crystalline increased plasma L-carnitine and milk + urine L-carnitine more than 40COAT and 60COAT (all $P < 0.05$). Carnitine supplementation increased carnitine concentrations in plasma, milk, and urine; however, the rumen protection did not provide additional increases in concentration.

Key words: L-carnitine, bioavailability, dairy cow

## Introduction

Fatty liver is a common metabolic disease that affects postpartum dairy cows. Depressed feed intake and increased energy demands of lactation lead to negative energy balance that stimulates fat mobilization, often in excess of the liver's oxidation capacity, causing liver lipid accumulation. L-carnitine stimulates hepatic fatty acid oxidation through increased transport of long chain fatty acids from the cytosol to mitochondria, and has been observed to decrease liver triglyceride accumulation during the transition period. L-carnitine is commonly degraded in the rumen, thus affecting its intestinal availability. An *in vitro* study estimated that 80% of dietary carnitine was degraded in rumen fluid after microbial adaptation. Studies implementing abomasal infusions have observed linear increases in urine, milk, plasma, and liver concentrations in response to infusions up to 6 grams/day. Rumen-protected products are intended to prevent degradation in

[1] Lonza, Inc., Allendale, NJ.

the rumen and increase the amount reaching the small intestine for absorption. In this experiment, two rumen-protected carnitine products were supplemented in the diets of mid-lactation Holstein cows and the L-carnitine concentrations in milk, urine, and plasma were determined to assess their relative bioavailability. Production responses including milk yield, milk components, and feed intake were also determined.

## Experimental Procedures

For this study, 56 mid-lactation Holstein cows (143 ± 72 days in milk) from the Kansas State University Dairy Teaching and Research Center were used in a randomized complete block design to determine the relative bioavailability of 2 rumen-protected carnitine products compared to crystalline (raw) carnitine. Cows were blocked by parity and level of milk production and then randomly assigned to 1 of 7 treatments within the block (8 cows per treatment). Cows were housed in a tie-stall facility and adapted for 4 days prior to 2 days of sample collection for baseline values. Following the 6-day baseline period, treatments were applied for a total of 8 days, with the final 2 days used for data and sample collection. The study was performed in 2 cohorts of cows.

Cows were milked 3 times daily at 0400, 1000, and 1800 h. The basal diet met estimated requirements for all nutrients and was fed as a total mixed ration twice daily (0600 and 1800 h). Animals had ad libitum access to feed in individual mangers and feed offered was adjusted daily to achieve 12-20% refusals. During the treatment period, the basal diet was top-dressed twice daily with the following treatments: a) control (no supplement); b) 3 grams raw carnitine; c) 6 grams raw carnitine; d) 5 grams carnitine with protection 1 (40% coating, 60% L-carnitine content); e) 10 grams carnitine with protection 1 (40% coating); f) 7.5 grams carnitine with protection 2 (60% coating, 40% L-carnitine coating); and g) 15 grams carnitine with protection 2 (60% coating). Supplementation rates were designed to provide 3 or 5 grams/day carnitine, regardless of the protection method.

During the 2-day collection periods, feed and water intake as well as milk yield were recorded. Ration samples were collected on each day of both baseline and treatment collection periods and composited for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY; Table 1). Health was monitored daily and one cow (7.5 grams of 60% coating) was removed from the study due to illness detected by a rapid decline in dry matter intake (DMI).

Over the course of the 48-hour collection period, urine and blood samples (coccygeal vein) were collected immediately prior to the initial feeding (2000 h), and 6, 12, and 18 hours after feeding. Blood samples were collected into $K_3$EDTA tubes and immediately placed on ice. Plasma was separated by centrifugation (1,500 × *g* for 15 minutes) and stored in microcentrifuge tubes at -20°C. Throughout the collection period, urine samples were composited by equal volumes into microcentrifuge tubes and frozen at -20°C until analysis of total carnitine and creatinine. Urine creatinine concentration and expected creatinine excretion of 29 mg/kg of BW daily was used to estimate daily urine volume. Two milk samples were collected at all 6 milkings during the 2-day collection periods, one used for milk component analysis by Heart of America Dairy Herd Improvement Association (Manhattan, KS) and the other frozen until carnitine analysis. Prior to analyses, the milk samples were composited in equal volumes by collection

period. Concentrations of total carnitine in plasma, milk, and urine were determined by an enzymatic radioisotope method.

Statistical analysis was performed using SAS (version 9.3, SAS Inst., Cary, NC). The mixed procedure was used to model treatment response variables using the covariate for the same variable from the basal period, the fixed effects of treatment and parity, and the random effect of block. Responses were assessed with 7 contrasts that assessed the linear and quadratic responses to raw, 40% coating, and 60% coating carnitine treatments as well as overall contrasts between raw and 40% coating, raw and 60% coating, and 40% vs. 60% coating treatments. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P \leq 0.10$.

## Results and Discussion

Production responses are summarized in Table 2. The 60% coating product linearly decreased DMI ($P = 0.02$) and raw carnitine tended to linearly decrease DMI ($P = 0.07$). The 60% coating product had a quadratic effect on milk fat percent ($P = 0.04$) and a tendency for a quadratic effect on milk yield ($P = 0.07$). Supplementation of raw carnitine had a quadratic effect on milk protein percent ($P = 0.04$). The 60% coating product tended to decrease milk protein percent ($P = 0.10$) and increase milk lactose percent ($P = 0.08$) compared to the 40% coating product. There were no treatment effects on milk yield, milk urea nitrogen, or yields of milk fat, protein, and lactose. This lack of response on milk yield and composition is consistent with the 6 g/day of abomasally infused raw carnitine found in a previous study.

Overall, there were no parity effects on plasma, milk, and urine carnitine concentrations, and only a tendency for a parity effect on total daily carnitine excretion ($P = 0.10$). Plasma samples were collected at 0, 6, 12, and 18 hours after feeding to assess diurnal variation; however, there was no effect of time on plasma carnitine concentrations ($P = 0.23$). Supplementation with raw carnitine or the 40% coating product increased plasma carnitine concentrations linearly ($P < 0.001$ and $P = 0.01$, respectively) whereas supplementation with the 60% coating product tended to linearly increase plasma carnitine ($P = 0.08$). Raw carnitine increased plasma carnitine compared to both the 40% coating product ($P = 0.03$) and the 60% coating product ($P < 0.001$). Urine carnitine concentrations also increased linearly with the raw ($P = 0.03$) and 40% coating products ($P = 0.02$). Effects on milk carnitine concentrations were more numerous, with linear effects across all sources, a quadratic tendency for the 40% coating product ($P = 0.08$). Raw carnitine increased milk carnitine concentration compared to the 60% coating product ($P < 0.01$) and tended to increased milk carnitine compared to the 40% coating product ($P = 0.08$). These effects were mirrored in daily milk carnitine output, with linear effects seen for all forms of supplementation, a significant difference between the 60% coating product and raw carnitine ($P < 0.01$), and a tendency for a difference between the 40% coating product and the raw carnitine ($P = 0.08$).

## Conclusion

Carnitine supplementation in the forms of raw carnitine and the 40% coating product were effective in linearly increasing carnitine concentrations. The subtle responses seen for the 60% coating product, which were significantly lower than that for raw carnitine

in several metrics, may have been due to over-encapsulation that hindered liberation of the carnitine and its absorption in the small intestine. Effective ruminal protection of L-carnitine while maintaining intestinal availability needs further investigation.

**Table 1. Ingredient and nutritional composition of the basal diet**

| Item | Value |
|---|---|
| Ingredient, % of dry matter | |
| Corn silage | 35.0 |
| Alfalfa hay | 14.2 |
| Wet corn gluten feed[1] | 27.3 |
| Cotton seed | 2.7 |
| Fine-rolled corn | 13.7 |
| Micronutrient premix[2] | 7.0 |
| | |
| Nutrient, % of dry matter (unless otherwise specified) | |
| Dry matter, % as-fed | 49.9 |
| Crude protein | 17.5 |
| Acid detergent fiber | 23.3 |
| Neutral detergent fiber | 36.3 |
| Lignin | 4.1 |
| Non-fiber carbohydrate | 33.0 |
| Starch | 16.2 |
| Crude fat | 4.9 |
| Net energy for lactation, Mcal/lb | 0.75 |

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

[2]Premix consisted of 54.6% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 14.8% limestone, 2.34% stock salt, 1.56 trace mineral salt, 0.16% potassium chloride, 10.9% sodium bicarbonate, 2.49% magnesium oxide, 0.24% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.12% Zinpro 100 (Zinpro Corp.), 0.26% selenium premix (0.06%), 0.16% vitamin A premix (30 kIU/g), 0.05% vitamin D premix (30 kIU/g), 1.56% vitamin E premix (48 kIU/g), 0.01% ethylenediamine dihydriodide premix, 0.07% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 1.95% XP Yeast (Diamond V, Cedar Rapids, IA), 0.97% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), and 7.8% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ).

**Table 2. Effect of carnitine supplementation on performance and milk production parameters**

| | | Raw | | 40 coat | | 60 coat | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | 3g | 6g | 5g | 10g | 7.5g | 15g | SEM[1] |
| DMI,[2] lb/day | 60.0 | 59.4 | 57.7 | 59.2 | 58.1 | 57.9 | 56.8 | 2.0 |
| Water intake,[3] liters/day | 128.2 | 131.4 | 128.4 | 136.8 | 120.6 | 125.8 | 125.9 | 5.6 |
| Milk, lb/day | 98.8 | 99.2 | 98.6 | 98.6 | 99.4 | 94.8 | 97.5 | 2.4 |
| Milk fat,[4] % | 3.66 | 3.53 | 3.47 | 3.50 | 3.47 | 3.31 | 3.48 | 0.11 |
| Milk protein,[5] % | 2.86 | 2.91 | 2.84 | 2.90 | 2.88 | 2.86 | 2.85 | 0.04 |
| Milk lactose, % | 4.92 | 4.94 | 4.92 | 4.92 | 4.89 | 4.95 | 4.95 | 0.03 |
| Milk somatic cell linear score | 1.61 | 1.46 | 1.96 | 1.22 | 1.53 | 2.01 | 1.21 | 0.48 |
| Milk urea nitrogen, mg/dL | 13.34 | 13.41 | 13.33 | 13.04 | 13.10 | 13.10 | 13.35 | 0.30 |
| Milk fat, lb/day | 3.62 | 3.42 | 3.44 | 3.51 | 3.35 | 3.15 | 3.42 | 0.15 |
| Milk protein, lb/day | 2.82 | 2.89 | 2.78 | 2.87 | 2.87 | 2.71 | 2.78 | 0.09 |
| Milk lactose, lb/day | 4.85 | 4.90 | 4.85 | 4.85 | 4.85 | 4.67 | 4.83 | 0.13 |

[1]Reported SEM is pooled across treatment groups.
[2]Linear effect of 60% coating product ($P < 0.05$).
[3]Quadratic effect of 40% coating product ($P < 0.05$).
[4]Quadratic effect of 60% coating product ($P < 0.05$).
[5]Quadratic effect of raw carnitine ($P < 0.05$).

**Table 3. Least squares means for concentrations of L-carnitine in plasma, milk, and urine from mid-lactation Holstein cows fed different amounts and sources of L-carnitine**

| | | Raw | | 40 coat | | 60 coat | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | 3g | 6g | 5g | 10g | 7.5g | 15g | SEM[1] |
| Plasma, μM[2,3,6,7] | 8.59 | 9.80 | 12.17 | 9.36 | 10.46 | 8.62 | 9.77 | 0.47 |
| Milk | | | | | | | | |
| μM[2,3,4,7] | 137.5 | 166.4 | 174.3 | 145.6 | 176.1 | 143.2 | 161.8 | 5.31 |
| g/day[2,3,4,7] | 0.97 | 1.17 | 1.22 | 1.05 | 1.22 | 0.99 | 1.15 | 0.03 |
| Urine | | | | | | | | |
| μM[2,3] | 9.63 | 10.37 | 11.47 | 10.02 | 11.63 | 9.93 | 10.74 | 0.62 |
| g/day[2,3,4,7] | 0.557 | 0.617 | 0.701 | 0.587 | 0.644 | 0.557 | 0.629 | 0.03 |
| Total excreted carnitine,[8] g/day[2-7] | 1.52 | 1.78 | 1.92 | 1.62 | 1.87 | 1.54 | 1.79 | 0.04 |

[1] Reported SEM is pooled across treatment groups.
[2]Linear effect of raw carnitine ($P < 0.05$).
[3]Linear effect of 40% coating product ($P < 0.05$).
[4]Linear effect of 60% coating product ($P < 0.05$).
[5]Quadratic effect of 60% coating product ($P < 0.05$).
[6]40% coating product vs. raw carnitine ($P < 0.05$).
[7]60% coating product vs. raw carnitine ($P < 0.05$).
[8]Milk plus urine carnitine.