J. Dairy Sci. 100:1–6
https://doi.org/10.3168/jds.2017-13093

Short communication: The mammalian lignan enterolactone is absorbed by newborn dairy calves fed enterolactone-enriched milk

C. P. Ghedini,* N. L. Whitehouse,* D. C. Moura,† A. S. Oliveira,† and A. F. Brito*[1]
*Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham 03824
†Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso–Campus Sinop, Sinop, MT, Brazil, 78557-267

ABSTRACT

Flaxseed is the richest source of the plant lignan secoisolariciresinol diglucoside, which is converted to the mammalian lignans enterolactone (EL) and enterodiol by the gut microbiota of ruminants and humans. Enterolactone has been associated with improved animal and human health due to its antioxidant and anticarcinogenic properties. The objective of this study was to determine the pharmacokinetics of EL in newborn dairy calves fed milk replacer or EL-enriched milk. We hypothesized that newborn Holstein calves fed EL-enriched milk would have greater area under the curve and plasma concentration of EL compared with those fed milk replacer. On d 5 of life, calves were administered 2 L of milk replacer (n = 10; low-EL treatment: 123 nmol/L of EL) or 2 L of EL-enriched milk (n = 10; high-EL treatment: 481 nmol/L of EL) during the morning feeding (0700 h). Blood samples were taken from the jugular vein before (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 h after oral administration of treatments. The area under the curve for the plasma concentration of EL was analyzed according to the trapezoidal rule between 0 and 12 h after treatment administration, and it was greater in high- (26 nmol/L × h) than low-EL calves (4.30 nmol/L × h). Similarly, the maximum concentration of EL in plasma was greater in high- (5.06 nmol/L) versus low-EL calves (1.95 nmol/L). Furthermore, the time after treatment intake to reach maximum plasma concentration of EL was faster in high- (4.31 h) compared with low-EL (4.44 h) treatment. Calves were able to absorb EL, indicating that EL-enriched milk can potentially be used as source of EL to pre-weaned ruminants.

Key words: enterolactone, dairy calf, lignan, pharmacokinetics, secoisolariciresinol diglucoside

Short Communication

Lignans are polyphenolic, phytoestrogenic compounds known to elicit a wide range of biological effects, including weak estrogenic, antiestrogenic, antioxidant, anti-inflammatory, anticarcinogenic, and cardioprotective activities (Adolphe et al., 2010; Imran et al., 2015). Flaxseed (*Linum usitatissimum* L.) is the richest source of the lignan secoisolariciresinol diglucoside (**SDG**), which is a precursor for the synthesis of the mammalian lignans enterolactone (**EL**) and enterodiol (**ED**) by the gut microbiota of humans (Gaya et al., 2016) and ruminants (Gagnon et al., 2009). Feeding incremental amounts of flaxseed meal to dairy cows linearly increased the milk concentration of EL, but no ED was detected in milk (Petit et al., 2009). Therefore, EL-enriched milk has the potential to be used as a source of EL for humans not only because milk is consumed by a large part of the world population, but also due to a poor and variable intake of plant lignans worldwide, including in the United States (de Kleijn et al., 2001).

Newborn calves often experience diarrhea, respiratory diseases, and oxidative stress, which contribute to high rates of morbidity and mortality during the first weeks of life (Inanami et al., 1999; Gaál et al., 2006; Uetake, 2013). In addition, poor colostrum quality is associated with low concentration of antioxidants (Maciej et al., 2015), suggesting that feeding EL-enriched milk to newborn and preweaned calves may be a viable strategy to mitigate oxidative stress. In newborn calves, suckling stimulates the reflex closure of the esophageal groove so that milk or milk replacer (**MR**) bypass the reticulo-rumen down to the abomasum. Thus, calves may be used as a model to make inferences about the pharmacokinetics of EL in simple-stomach mammals including humans. We hypothesized that compared with calves fed MR, those fed EL-enriched milk would have increased area under the curve (**AUC**), as well as greater maximum plasma concentration ($\mathbf{C_{max}}$) of EL and faster time to reach C_{max} ($\mathbf{T_{max}}$). The objective of this study was to determine the pharmacokinetics of EL from MR or EL-enriched milk consumed by newborn Holstein calves.

Received April 29, 2017.
Accepted June 17, 2017.
[1]Corresponding author: andre.brito@unh.edu

This experiment was conducted at the University of New Hampshire Fairchild Dairy Teaching and Research Center (Durham, NH) from May to August 2015 and was approved by the University of New Hampshire Institutional Animal Care and Use Committee (protocol no. 15303). Twenty Holsteins calves (n = 10 males and 10 females) were used from birth to d 7 of life. Calves were removed from dams immediately after birth and before nursing, weighed, navel-dipped with 7% iodine (vol:vol), and placed in individual pens (1 × 2.15 m) located in an enclosed calf room. The 10 calves (n = 6 males and 4 females) born from multiparous cows received 4 L of colostrum using nipple bottles, with the first 2 L fed immediately after they were moved to the pens and the remaining 2 L within the first 24 h of life. The 10 calves (n = 4 males and 6 females) born from primiparous cows were fed 4 L of stored colostrum from multiparous cows when available or colostrum replacer (Ultra Start 150 Plus, Milk Products LLC, Chilton, WI) split in 2 daily allotments of 2 L each as done for calves born from multiparous cows. When colostrum or colostrum replacer was completely refused or not fully consumed voluntarily within approximately 15 min after offering the meal, refusal was administered via an esophageal tube to ensure uniform consumption among animals. Calves had free access to water and no access to starter grain while enrolled in the study.

From d 2 to 4 of life, nipple bottles were used to feed 4 L/d of nonmedicated MR (Calf Care All-Milk 22–20, Poulin Grain, Newport, VT) to all calves in 2 daily allotments (0700 and 1900 h) by mixing 300 g of MR powder plus 2 L of warm tap water following standard operation procedures of our dairy facility. On d 5 of life, calves were randomly assigned to 1 of 2 treatments: low milk EL (**L-EL**; n = 5 females and 5 males) or high milk EL (**H-EL**; n = 5 females and 5 males). Calves assigned to the H-EL treatment had MR substituted for 2 L of EL-enriched milk during the morning feeding on d 5 of life, whereas L-EL calves continued to receive 2 L of MR per feeding until d 7. Administration of MR resumed at 1900 h on d 5 for H-EL calves and continued through d 7. All calves completely consumed the MR or EL-enriched milk within 5 min after the meal was offered. The EL-enriched milk used in the current study was collected over 3 consecutive afternoon milkings (total = 28 kg) from 1 multiparous Jersey cow fed a TMR containing (DM basis) 15% flaxseed meal and 12% liquid molasses (Ghedini et al., 2016). Milk was stored at −20°C in 3-L plastic bottles for at least 90 d before being administered to the calves using nipple bottles. The concentration of EL (mean ± SD) averaged 123 ± 6.53 nmol/L and 481 ± 65 nmol/L for MR and EL-enriched milk, respectively. The health status of all calves was evaluated daily while they were enrolled in the study (i.e., 7 d) by monitoring signs of illness including lethargy, weakness, decreased appetite, fever, abnormal fecal consistency, cough, ocular or nasal discharge, and drooping ears using a calf health scoring chart (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf; accessed Mar. 25, 2017).

Blood samples were taken from the jugular vein on d 5 of life before (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 h after oral administration of MR or EL-enriched milk using 10-mL Vacutainer tubes containing K_3-EDTA (Covidien, Minneapolis, MN). After collection, tubes were immediately transported to the laboratory and centrifuged (3,300 × g) for 20 min at 4°C. Aliquots (1.8 μL) of plasma were stored at −80°C until EL analysis. Samples (40 mL) of MR and EL-enriched milk were taken during the morning feeding on d 5 and stored at −20°C for later EL analysis. Enterolactone in plasma, MR, and EL-enriched milk was extracted and hydrolyzed [β-glucuronidase/arylsulfatase from *Helix pomatia* (Roche-Diagnostics, Laval, QC, Canada)] according to procedures described previously (Gagnon et al., 2009). Enterolactone was analyzed colorimetrically (UV/visible spectrophotometer set at a wavelength of 405 nm) in quadruplicates using a competitive enzymatic immunoassay (assay kit no. 500520, Cayman Chemical Co., Ann Arbor, MI) that recognizes both enantiomeric forms of EL. Plasma EL values were corrected by baseline concentrations of EL (i.e., 0-h blood sampling).

The AUC between 0 and 12 h ($AUC_{0–12\ h}$) after oral administration of MR or EL-enriched milk on d 5 of life was determined according to the trapezoidal rule (Phillips and Taylor, 1973). Both $C_{max\ (0–12\ h)}$ and $T_{max\ (0–12\ h)}$ were determined from individual baseline corrected plasma concentration time curves (Maciej et al., 2015). The apparent efficiency of absorption (**AEA**) of EL between 0 and 12 h was calculated assuming no change in plasma volume from d 1 to 5 of life using the following equation (Quigley et al., 1998):

$$AEA_{0–12\ h}\ (\%) = [\text{plasma EL (mg/L)} \times \text{BW (kg)} \times 0.092 \div \text{EL intake (mg)}] \times 100.$$

The experiment was analyzed as a randomized complete block design with repeated measures over time [i.e., $AUC_{0–12\ h}$, $C_{max\ (0–12\ h)}$, and $T_{max\ (0–12\ h)}$] using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Calves were blocked by date of birth yielding a total of 10 blocks with 2 animals/block. The random effect of block, as well as the fixed effects of treatment, time of blood sampling, and covariate measurements (i.e., initial BW and 0-h plasma concentra-