

# Effect of Milk Fraction on Concentrations of Cephapirin and Desacetylcephapirin in Bovine Milk after Intramammary Infusion of Cephapirin Sodium

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## Introduction

Treatment of clinical mastitis accounts for a substantial proportion of antimicrobial use on dairy farms. Intramammary (IMM) antibiotics are a mainstay of treatment for clinical mastitis, especially episodes caused by streptococci or staphylococci. For therapy to be successful, the antimicrobial treatment must attain and maintain an effective concentration at the site of infection. Accurate pharmacokinetic data are essential for determining appropriate treatment regimens and withholding times, especially when antimicrobials are used off-label, because milk is withheld from sale until antimicrobial residues drop below the tolerance concentration. Some studies use foremilk or total milk collected during milking for antimicrobial quantification, whereas many studies do not report sample type. Because antimicrobials distribute unevenly in the mammary gland, and because milk fractions differ in protein and fat concentrations which influence distribution, we hypothesized that antimicrobial concentrations differ in milk fractions after IMM infusion. Therefore, our objective was to compare antimicrobial concentrations in three fractions of milk 8 or 12 hours after administering an IMM antimicrobial to lactating dairy cows.

## Materials and Methods

Six healthy, multiparous Holstein cows were enrolled, three with low milk yield (< 55 lb/d) and three with high milk yield (≥ 80 lb/d). One rear mammary gland/cow was infused with 200 mg cephapirin sodium after each of two milkings. Milk samples from the treated glands were collected at the next milking. Samples included 5 mL of foremilk, 20 mL of milk collected from the bucket of the quarter milking device (bucket milk), and 5 mL of strippings collected by hand immediately after milking. Samples were held at -70 °C until assayed for concentrations of cephapirin (CEPH) and its active metabolite desacetylcephapirin (DAC) using HPLC with tandem mass spectrometry. Concentrations of CEPH and DAC were summed to produce total antimicrobial (TA)

concentration. The study was repeated three times/cow, using different milking frequencies and treatment intervals in a randomized crossover design, resulting in 6 sets of fraction samples/cow. Cows were milked every 12 hours and treated at 0 and 12 hours, or milked every 8 hours and treated at 0 and 8 or 0 and 16 hours. Cows were acclimated to the milking frequency for 48 hours before beginning each treatment phase, and a 4 days washout period was provided between the last infusion and next acclimation period. Data were analyzed by mixed models ANOVA, with cow as a random factor.  $P < 0.05$  was considered significant.

## Results

CEPH concentration was higher in foremilk (geometric mean 44.2 µg/mL) than bucket milk (15.7 µg/mL) or strippings (18.5 µg/mL), with no difference between bucket milk and strippings. The same was true for DAC (59.5, 23 and 30.2 µg/mL, respectively) and TA (106.4, 40.5 and 50.9 µg/mL, respectively). Concentrations of CEPH, DAC and TA were higher in cows milked every 8 h, regardless of treatment regimen, than those milked every 12 h due to the shorter interval between treatment and sampling. Milk production did not affect antimicrobial concentrations.

## Significance

Higher antimicrobial concentrations in foremilk than bucket milk suggest that pharmacokinetic data and residue test results based on foremilk samples may be misleading. Concentrations in strippings were more representative of those in bucket milk, at least for cephapirin sodium. The relationship between milk fraction and antimicrobial concentration should be investigated for other classes and formulations of antimicrobials, in cows with mastitis, and at longer intervals after dosing, to determine if findings can be generalized. In the meantime, it is essential that pharmacokinetic and residue studies report how milk was collected and what fraction was analyzed.