DETERMINATION OF TOTAL POTENTIALLY AVAILABLE NUCLEOSIDES IN BOVINE COLOSTRUM AND MILK

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INTRODUCTION

Nucleosides and nucleotides are compounds of critical importance to cellular function. Dietary sources of nucleotides are considered conditionally essential for continued optimal metabolic function. Dietary nucleotides are ingested in the form of nucleoproteins, polymeric nucleotides (nucleic acids) and nucleotide adducts as well as free nucleotides.

In order to determine the total potentially available nucleosides (TPAN), an analytical protocol to characterise the contributions of different molecular nucleoside sources to infant nutrition was developed (Leach et al. Am. J. Clin. Nutr. 1995, 61, 1224–1230). The analytical method uses a number of enzymatic treatments and incorporates combinations of nuclease, pyrophosphatase and phosphatase enzymes into the sample preparation. The development of this protocol has been an important contribution to further understanding the distribution of nucleosides and nucleotides and their implications for infant nutrition.

Bovine milk is almost exclusively used in the manufacture of infant formula intended to substitute for human breast milk, and since the levels of TPAN in bovine milk have not been previously reported, the purpose of the current study was to evaluate bovine milk TPAN levels and variation over the first month of lactation.

ANALYTICAL TECHNIQUE

Sample Collection
Samples from a winter-milk herd were collected over a 1 month period in late March 2008 and samples from a summer-milk herd were collected over a 1 month period in early August 2009. Collected samples were initially refrigerated at 4 °C, then taken to the laboratory where endogenous enzymes were chemically inactivated prior to storage at < -15 °C.

Sample Preparation
Each sample was pooled, then split into four 5 mL sub-samples, to each of which internal standard (10 µg, 5-methylcytidine) was added, and then each sub-sample was subjected to a different enzymatic treatment.

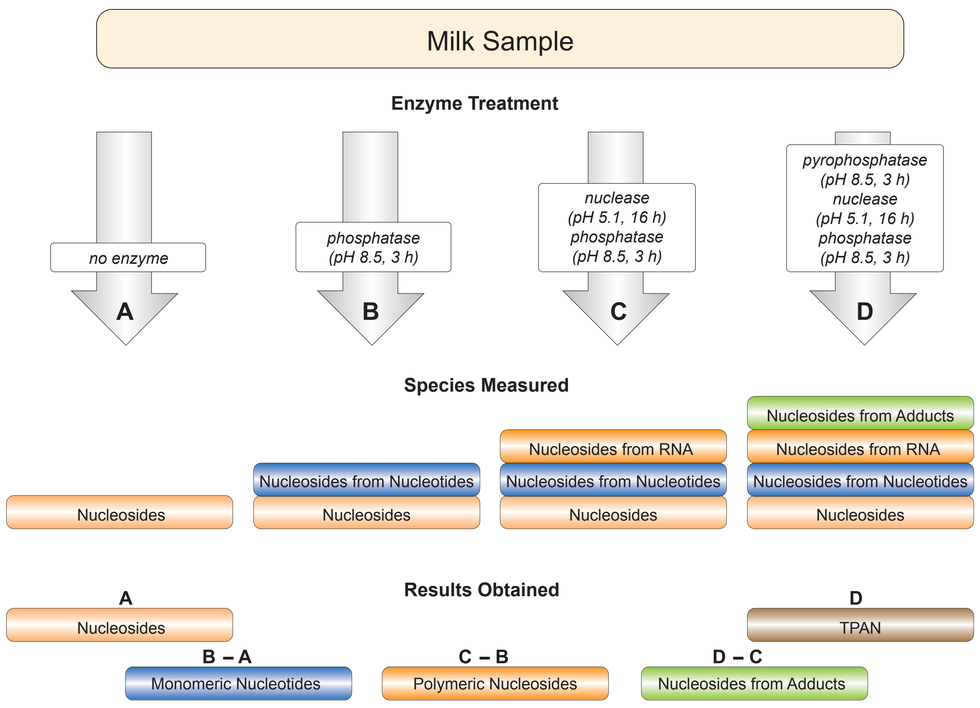

Figure 1: Schematic of TPAN experiments

Solid Phase Extraction
Clean-up of enzymatic extracts was achieved by solid phase extraction using a phenylboronate affinity gel (Bio-Rad), whereby nucleosides are covalently bonded to the gel at high pH, and interferences were then removed with two washings in high pH buffer. The nucleosides were then eluted from the affinity gel at low pH with the addition of phosphoric acid, and filtered ready for analysis.

Chromatographic Analysis
Column: Prodigy C_{18} 5 µm, 4.6 x 150 mm (Phenomenex)
Mobile Phase: (A) $NaCH_3COO$ (0.05 M), pH = 5.6, (B) MeOH (100%)
Flowrate: 0.7 mL/min with low-pressure gradient mixing (A) & (B)
Detection: Photo-diode array 210–300 nm, quantitation at 260 nm
Quantitation: Internal standard technique (5-methylcytidine)

The nucleoside results for each of the four sub-samples allowed the contributions of the different forms (nucleosides, nucleotide adducts, monomeric and polymeric nucleotides) to TPAN to be calculated.

RESULTS AND DISCUSSION

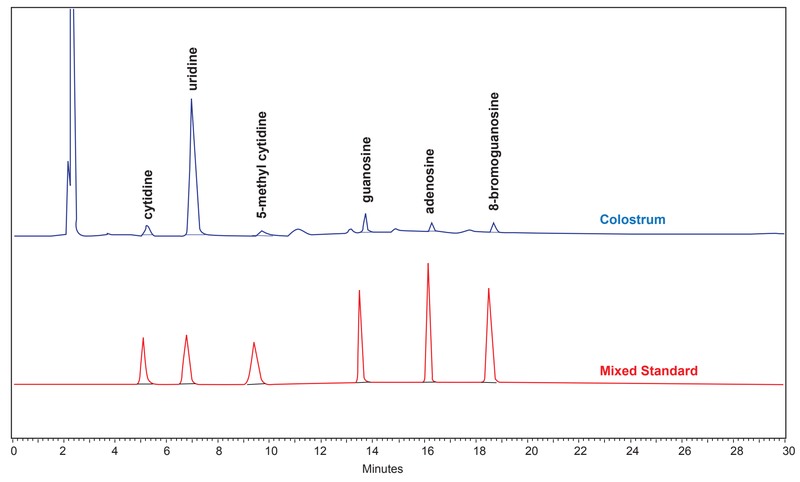

Figure 2: Chromatograms of a mixed standard and colostrum sample

The absolute concentrations indicated a distinct difference between the two herds, although the general trends were the same. High levels of TPAN were found in colostrum, with a decrease in their concentrations as lactation progressed.

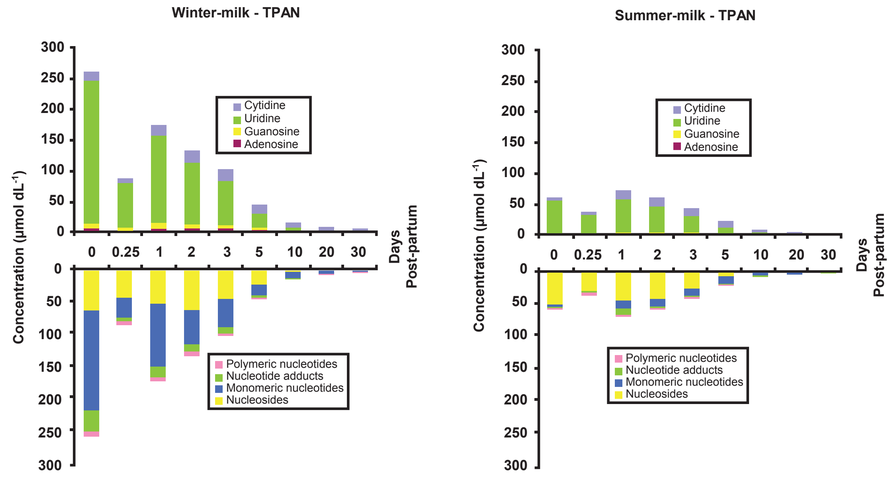

Figure 3: TPAN in bovine milk over the first month of lactation

A comparison of the two herds showed little difference in total free nucleoside content as lactation progressed. Differences in colostral monomeric nucleotide levels between the herds were seen, with the winter-milk herd containing 5–10 times the levels of the summer-milk herd although by the fifth day, nucleotide levels decreased to similar levels between the two herds. The levels of monomeric nucleotides measured in this study were generally higher than those reported previously (Gill and Indyk, Int. Dairy J. 2007, 17, 596–605), most likely due to a significant contribution from multiple phosphorylated forms, which the TPAN analytical method aggregates as a single value.

The pyrimidines (cytidine and uridine) differed markedly from each other through lactation. Whereas the quantities of cytidine and cytidine nucleotides were relatively constant throughout, uridine and uridine nucleotides levels varied considerably. In contrast, concentrations of the purines (guanosine and adenosine) in bovine milk were more consistent through lactation. Purine nucleosides and nucleotides made a relatively small contribution to TPAN (6–20%).

CONCLUSIONS

Bovine milk samples from two herds were studied over the course of the first month of lactation, and total potentially available nucleosides were determined.

- Uridine and uridine nucleotides were the major contributor to TPAN in early colostrum
- Differences in TPAN concentrations between summer milk and winter-milk herds were largely attributable to variability in uridine and nucleotide concentrations.
- TPAN concentration decreased as lactation progressed, as did each of the contributing forms.

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