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Nucleotides and nucleosides in ovine and caprine milk during lactation

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ABSTRACT

The aim of this study was to determine the nucleoside and nucleotide content in ovine and caprine milks at the colostral, transitional, and mature stages of lactation. Samples from 18 dairy sheep and 18 dairy goats were collected at 1, 2, 3, 4, 5, and 15 d postpartum. Separation and quantitation of the 5′-nucleotides (NT) and the nucleosides (NS) was performed by reverse phase HPLC. For each compound measured, considerable interindividual variation was recorded in both species of milk. The total NS content ranged from 57 to 132 μmol/L and from 54 to 119 μmol/L in ovine and caprine milk, respectively. The major NS identified in both species of milk was uridine, representing more than 60% of the total NS pool. The mean levels of inosine and guanosine were comparable between ewe and goat milk. Instead, the mean level of cytidine across the sampling period was much higher in ewe milk (11.9 μmol/L compared with 4.5 μmol/L in goat milk) and exhibited a peak value on the fourth day of lactation. The adenosine content was at least 3-fold higher in caprine milk compared with its ovine counterpart. The total NS and orotic acid contents did not differ significantly between the 2 species. However, in the case of total NT content, interspecies differences were significant, with NT levels ranging from 294 to 441 μmol/L in ovine milk and from 166 to 366 μmol/L in caprine milk. The NT content in colostrum (1–3 d) of both species was higher than in mature milk (15 d), and uridine monophosphate was the dominant NT in all samples.
Key words: nucleotide, nucleoside, sheep milk, goat milk

INTRODUCTION

As nutrition science moves beyond the study of essential nutrients, considerable research interest is focused on minor components of the non-protein–nitrogen fraction of milk that may be able to transmit biochemical messages with significant health implications (Michaelidou and Steijns, 2006; Michaelidou, 2008). As such, nucleotides (**NT**) have attracted particular scientific attention because they are ubiquitous intracellular compounds of crucial importance to cellular function and metabolism. The work conducted by Cosgrove (1998) on the biological role of these compounds indicated their relation with the following 5 main areas: immune function, iron absorption, lipid metabolism, intestinal flora, and intestinal and hepatic morphology and function. Nucleotides can be synthesized endogenously and are, therefore, not considered essential nutrients (Sánchez-Pozo and Gil, 2002). However, investigations in human and animal models suggest that dietary NT may become essential when the endogenous supply is insufficient for normal function, even though their absence from the diet does not lead to a classic clinical deficiency syndrome. Conditions under which these nutrients may become essential include certain disease states, periods of limited nutrient intake or rapid growth, and the presence of regulatory or developmental factors that interfere with full expression of the endogenous synthetic capacity. Under these conditions, the dietary intake of the nutrient spares the organism the cost of de novo synthesis or salvage and may optimize tissue function (Carver, 1999).

During the last 3 decades, numerous studies have dealt with the occurrence of NT and their metabolites in human milk, given the functional role that these components play in neonatal nutrition (Gil and Sanchez-Medina, 1982; Janas and Picciano, 1982; Leach et al., 1995; Thorell et al., 1996; Duchén and Thorell, 1999). However, fewer studies have been devoted to bovine NT profile (Tiemeyer et al., 1984; Schlimme et al., 1997, 2000; Ferreira, 2003) and an even more limited number to the content and distribution of NT and nucleosides (**NS**) in ovine and caprine milk (Schlimme et al., 1997; Martin et al., 2005).

Although about 84% of milk worldwide is produced by cows (IDF, 2008), the contribution of milk from other domesticated animals to the survival and well-being of people around the world is immense and invaluable, especially in areas where cow breeding is difficult because of adverse environmental conditions.

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Greece is one of the leading ovine and caprine milk producers in the Mediterranean region and the leading producer in Europe. Caprine and ovine milk compose more than 60% of Greek milk production, reaching the amount of 1.25 million tonnes annually (IDF, 2008). As a consequence, these milk species are considered a fundamental and indispensable part of the Greek and Mediterranean diet.

The potential value of using ovine or caprine milk or the respective colostral fractions in clinical nutrition is gathering momentum because of the important role their nonpeptide trophic factors, such as nucleotides and nucleosides, could play in homeostatic regulation. In particular, these factors can help to maintain gastrointestinal mucosal mass and modulate the immune system via multiple mechanisms (e.g., altering intestinal flora and influencing the actions of growth factors; Playford et al., 2000). It is worth noting that because of the bio- and trophochemical properties of dietary nucleotides and nucleosides, the European Commission has permitted the use of supplementation with specific ribonucleotide salts in the manufacture of infant and follow-on formulas (EC, 1996). Therefore, besides the attractive value to different clinical situations, the opportunity to use ovine and caprine colostrum and milk to develop problem-oriented supplementation packages for preterm neonates and infants that are small for gestational age remains an appealing area of research.

The present study was motivated by the nutritional significance of these compounds for specific population groups and the economic impact of ovine and caprine milk production in Greece and other Mediterranean countries. Thus, the objective was to identify and quantify free NT and NS in ovine and caprine milk from indigenous Greek breeds sampled at different stages of lactation.

MATERIALS AND METHODS

Sample Preparation

Colostrum and milk were collected from 18 ewes of the Serron breed and 18 local-breed goats. Sampling was performed at 1, 2, 3, 4, 5, and 15 d after parturition. Samples were flushed with N_2 and frozen immediately after collection and were then stored at −25°C until analyzed.

Sample preparation was performed as follows. Equal volumes of milk and 13% (wt/vol) perchloric acid (Merck, Darmstadt, Germany) were mixed for 10 min at room temperature using a magnetic stirrer. Before mixing, colostrum samples from 1 and 2 d postpartum were diluted 1:1 with distilled water. The precipitate was separated by centrifugation at 7,000 × *g* for 15 min at 4°C. The pH of 25 mL of the supernatant was slowly adjusted to 4.00 using 5 *M* KOH (Panreac, Barcelona, Spain) and the volume was brought to 50 mL using double-distilled water. Samples were kept for 1 h in an ice bath. A portion of 2.5 mL was then filtered through 0.2-μm cellulose acetate filters (Alltech Assoc. Inc., Deerfield, IL) and used for analysis. All reagents employed for the extraction step were of analytical grade.

Standard Solutions

Analytical grade nucleotides [adenosyl-5′-monophosphate (**AMP**), cytidyl-5′-monophosphate (**CMP**), uridyl-5′-monophosphate (**UMP**), and guanosyl-5′-monophosphate (**GMP**)], nucleosides (cytidine, uridine, inosine, guanosine, and adenosine), and orotic acid (Sigma, St. Louis, MO) were used for preparation of standard solutions. The solutions were filtered through 0.2-μm cellulose acetate filters (Alltech Assoc. Inc.) and stored at 4°C.

Chromatographic Analysis

Separation and quantitation of the 5′-nucleotides and the NS was performed by reverse phase-HPLC using a binary solvent system (LKB, Bromma, Sweden) in conjunction with a Nucleosil C_{18} column (120–5 μm, 250 × 4 mm; Macherey-Nagel, Düren, Germany) and a guard column (40 × 4 mm). Solvent A was 0.15 *M* KH_2PO_4, pH 4.00, and solvent B was 25% (vol/vol) acetonitrile in solvent A. The elution was conducted at room temperature at a flow-rate of 0.8 mL/min, with a linear gradient from 0 to 20% (vol/vol) solvent B for 20 min followed by a linear gradient from 20 to 100% (vol/vol) solvent B for 5 min, a linear gradient from 100 to 0% (vol/vol) solvent B for 1 min, and an isocratic elution with solvent A for 9 min. The absorbance of the eluate was monitored at both 254 and 278 nm using a programmable UV/visible detector (Fasma 525, Linear Instruments, Reno, NV), which was linked to a data acquisition and processing system (Nelson Analytical Inc., Paramus, NJ). The determination of orotic acid was performed using the same system under isocratic conditions for 5 min, with the mobile phase being 0.1 *M* trisodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$), pH 6.50, containing 8 mL/L of acetonitrile; the flow-rate was 0.8 mL/min, and the absorbance was monitored at 278 nm.

All solvents were filtered through 0.45-μm Nylon 66 filters (Alltech Assoc. Inc.) before chromatographic analysis. Each compound was identified by its retention time when coinjected with the standards. Quantitation was carried out by use of external standard calibration; 5 concentrations were used for plotting the calibration