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Polyamine profile in ovine and caprine colostrum and milk

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ABSTRACT

The objective of this study was to monitor the *post-partum* variation of polyamine content, in ovine and caprine milk, from indigenous Greek breeds. Twenty samples of ewe and 20 samples of goat colostrum and milk were collected at the 1st, 2nd, 3rd, 4th, 5th and 15th day *post-partum*. Putrescine, spermidine and spermine were measured as dansylated derivatives by high-performance liquid chromatography. Putrescine was the least concentrated of these substances in both milk types. Spermidine was the prevailing polyamine in caprine samples, reaching levels up to 4.41 μmol/l on the 3rd day *post-partum*. In ovine milk, the profile of the mean concentrations showed greater levels of spermine than spermidine, except for the 5th day *post-partum*. These data suggest that goat colostrum and ewe milk (15th day) could be considered as good natural sources for these bioactive growth factors, and may become useful raw materials for designing tailored dairy products for specific population groups.

1. Introduction

The evolution of nutrition sciences beyond the study of essential nutrients has begun to provide strong scientific evidence reinforcing the belief that a personalised dietary intervention approach for health promotion and disease prevention could be a feasible and effective strategy to improve human health. Among other bioactive constituents, there is increasing interest in the naturally occurring polyamines, putrescine, spermidine and spermine, compounds with a broad range of bio-functionality (Atiya Ali, Poortvliet, Strömberg, & Yngve, 2011; Atiya Ali, Strandvik, Palme-Kilander, & Yngve, 2013; Atiya Ali, Strandvik, Sabel, et al., 2013; La Torre, Saitta, Potorti, Di Bella, & Dugo, 2010; Plaza-Zamora et al., 2013; Salimei & Fantuz, 2012; Silanikove, Leitner, Merin, & Prosser, 2010).

The polyamines spermidine and spermine, as well as their diamine precursor putrescine, are ubiquitous constituents of all prokaryotic and eukaryotic cells and are considered as indispensable in various physiological/metabolic processes of cell differentiation and growth (Löser, 2000). Some of their effects have been linked to their role in stabilising the negative charges of DNA and of the chromatin structure, the regulation of several transcriptional factors and protein synthesis (Larqué, Sabater-Molina, & Zamora, 2007). Apart from participating in cellular growth and differentiation, they are also involved in the regulation of inflammatory, digestive, immunological and immunoallergic responses, since they mediate the action of known hormones and growth factors (Bardócz & White, 1999; Eliassen, Reistad, Risøen, & Rønning, 2002; Löser, 2000). It should also be emphasised that, under circumstances of rapid growth and cell proliferation (as in newborns or during the recovery of injured tissues), the polyamine body pool must be supported by exogenous dietary sources. Organs and systems with a high cell turnover are especially dependent on the supply of dietary polyamines (Gugliucci, 2004; Kalač & Krausová, 2005; Larqué et al., 2007). As reviewed by Deloyer, Peulen, and Dandrifosse (2001), polyamines provided by food have a potential role in growth and development of the digestive system in neonatal mammals, and also seem necessary for the maintenance of normal growth and general properties of the adult digestive tract. Dietary polyamines might, in addition, become important with ageing, as cell proliferation slows with age and ornithine decarboxylase activity also decreases (Nishimura, Shiina, Kashiwagi, & Igarashi, 2006).

Milk provides an easily accessible matrix, rich in a large variety of essential nutrients, such as vitamins, minerals and proteins, with a well-balanced amino acid profile. Moreover, milk contains unique bioactive substances that may be able to transmit biochemical messages with significant health implications. The nutritional contribution of milk and dairy products is important in supporting good body function, especially during the periods of rapid growth (Michaelidou, 2008; Michaelidou & Steijns, 2006). Apart form customary dairy products, dairy derived ingredient preparations and functional dairy foods appear to be a novel attractive dietary

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option, having the advantage of being "natural" products, which might result in greater acceptance and compliance with nutritional guidelines (Playford, MacDonald, & Johnson, 2000; Regester & Belford, 1999).

Over several decades, research has focused on bovine milk, which is among the most rigorously described biomaterial matrices with respect to molecular species composition (Food and Agriculture Organization of the United Nations [FAO], 2013). Over the last few years, there has been a steady increase in studies concerning the functional and nutritional attributes of milk from other mammalian species (Cheng, Li, Ge, & Xing, 2006; La Torre et al., 2010; Salimei & Fantuz, 2012). There is increasing awareness of the nutritional and health aspects of milk from small ruminants, especially goat milk (Gill, Indyk, & Manley-Harris, 2012; Park & Haenlein, 2006; Silanikove et al., 2010; Zervas & Tsiplakou, 2013). Several studies support the idea that goat's milk is of a higher nutritional quality than cow's milk, in terms of higher protein efficiency ratio, protein digestibility, nitrogen balance and food conversion ratio (Almaas et al., 2006; López-Aliaga, Diaz-Castro, Alférez, Barrionuevo, & Campos, 2010). However, research data on the minor bioactive components of ewe and goat's milk are still quite limited. It is important to further explore the dietary significance of these milk types, since they are considered as a fundamental and indispensable part of the economy in southern European countries, such as Greece.

The aim of this work was to determine the polyamine concentrations in ovine and caprine milk, from indigenous Greek breeds, in the early *post-partum* period (first 5 days) and on the 15th day. Determination of the polyamine pool in both milk types will provide valuable scientific data concerning the dietary aspects of these two milk categories, aiming towards the possibility of using them in manufacturing quality-oriented dairy products and supplementation packages, in cases where cellular growth and differentiation is a fundamental target.

2. Materials and methods

2.1. Sample preparation

The study was performed on 20 Chios sheep and 20 goats of a local-breed, called "Eghoria". All lactating animals were in good health and had no illness during pregnancy. Fresh colostrum and milk samples were collected at days 1, 2, 3, 4, 5 and 15 of lactation. Sample collection and sample preparation before analysis was according to standardised procedures to minimise endogenous enzymic action (polyamine oxidases) which could lead to reduction of polyamine concentrations during storage. In fact, all samples were flushed with N_2 and frozen immediately after collection and stored at −20 °C until analysed.

2.2. Analytical procedure

Sample preparation was performed as described by Romain, Dandrifosse, Jeusette, and Forget (1992). The milk samples were left overnight in the refrigerator (4 °C) to thaw. Afterwards, they were vortexed for 30 s. Solid sulfosalicylic acid (20 mg) was added to 300 μl of milk and 300 μl of distilled water, and the samples were held overnight at −20 °C for protein precipitation. After centrifugation at 10,000*g* for 15 min, aliquots of supernatants (200 μl) were used for derivatisation.

The dansylation procedure was carried out as follows: a 200 μl sample was pipetted into a 13 × 100 mm screw-capped culture tube. A portion of 8 μl of 20 nmole/ml solution of 1,6-diaminohexane (internal standard), 292 μl of 0.5 M carbonate buffer (pH 9.2), 100 mg of anhydrous potassium carbonate and 500 μl of 2 mg/ml dansyl chloride in acetone were added and thoroughly mixed by vortexing for 30 s. Polyamine dansylation was accomplished by allowing the samples to remain at room temperature overnight. Afterwards, 5 ml of n-heptane was added to each tube and the samples were mixed. The organic layer was pipetted into a clean tube and the solutions were placed in a water bath (30 °C) and dried by a stream of nitrogen. Reconstitution of the samples was achieved with 200 μl of HPLC-grade methanol and 10 μl of glacial acetic acid.

2.3. Reagents and solutions

Putrescine dihydrochloride (98%), spermidine trihydrochloride (98%), spermine tetrahydrochloride (95%), 1,6 diaminohexane, anhydrous potassium carbonate, solid sulfosalicylic acid and dansyl chloride were purchased from Sigma–Aldrich Chemie GmbH (Taufkirchen, Germany). 1-Heptanesulphonic acid, acetonitrile and methanol (HPLC-grade), n-heptane, glacial acetic acid and acetone were obtained from Panreac Quimica S.L., (Barcelona, Spain).

2.4. High performance liquid chromatography analysis

The polyamines were determined as dansylated derivatives by reverse phase high-performance liquid chromatography (RP-HPLC). The RP-HPLC binary analytical system (LKB, Bromma Sweden) was equipped with a Nucleosil C18 wide pore analytical column (5 mm, 30 nm, 250 × 4 mm; Macherey–Nagel, Düren, Germany) and a guard column (40 × 4 mm). Samples were introduced using a Rheodyne injector (Rheodyne 7125, Rheodyne Inc., Cotati, CA, USA) equipped with a 20 μl injection loop. Eluent A was 20 mM sodium 1-heptanesulphonate and 20 mM acetic acid solution in water. Eluent B was HPLC-grade acetonitrile. Dansylated polyamines were eluted at a flow rate of 1 ml/min using a gradient: 0–9 min, 57% B; 9–16 min, 57–100% B; 16–20 min, 100% B; 20–21 min, 100–57% B; 21–25 min, 57% B. Compounds were detected by UV absorbances at 365 nm and 485 nm.

2.5. Analytical quality control

Verification of the analytical method was performed with matrix spiking by standard samples of high purity putrescine, spermidine and spermine reagents. Recovery values were 98% for spermine, 96% for spermidine and 90% for putrescine. An internal standard (1,6-diaminohexane) was included in all analyses performed, to detect any precision problems caused by the sample preparation protocol or injection volume variations.

2.6. Statistical analysis

The experimental data were analysed by the ANOVA method according to the linear model, which involves one factor between (animal species: sheep and goats) and one factor within experimental units (repeated measures at six sampling times *post-partum*); this analysis is equivalent to the analysis of a split-plot design (Gomez & Gomez, 1984). The normality and homogeneity of variance assumptions were tested prior to analysis of variance. Due to violations of the aforementioned assumptions, all measured concentrations (X, in μmol/l) were transformed as $\log_{10}(X + 1)$. Differences between means were compared using the LSD criterion. All statistical analyses were performed by the SPSS ver.15.0 statistical software. The significance level of all hypothesis testing procedures was preset at $a = 0.05$. Zero order correlation coefficients (Pearson's r values) were calculated in order to test the associations between polyamine concentrations.