Investigation of concentration of thiocyanate ion in raw cow’s milk from China, New Zealand and the Netherlands

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1. Introduction

The lactoperoxidase system (LPS) is a naturally occurring enzymatic antibacterial system in milk. Lactoperoxidase facilitates the oxidation of halides and thiocyanate by hydrogen peroxide, to produce a range of antimicrobial compounds (Björck, 1978; Fweja, Lewis, & Grandison, 2007). In countries where adequate refrigeration equipment is not accessible, and where it is permitted by regulation, the LPS may be artificially activated by means of a controlled increase in the concentrations of the two natural milk components hydrogen peroxide and thiocyanate ion (using sodium percarbonate and sodium thiocyanate) to temporarily inhibit pathogenic microorganisms (CAC, 1991). When used as intended, the level of thiocyanate added to milk for treatment is within the natural variation in thiocyanate found in milks, and the levels of thiocyanate in LPS treated milk do not present a food safety concern (FAO/WHO, 2005).

However, misuse of the LPS preservation system by an excessive addition of sodium thiocyanate may give rise to certain health hazards. Toxicity often occurs at plasma thiocyanate concentrations above 120 mg/L. Plasma concentrations in the order of 200 mg/L have been reported in fatalities (FAO/WHO, 2005), lower doses (4.8–6.4 mg/L) of thiocyanate have been shown to competitively inhibit iodide uptake by the sodium-iodide symporter in the thyroid gland (Dohan, De la Vieja, & Carrasco, 2000; FAO/WHO, 1990; Green, 1978; Wyngaarden, Wright, & Ways, 1952). Iodide is a key component in the production of thyroid hormone; sufficient suppression of iodide uptake into the thyroid is known to diminish production of thyroid hormone. Thyroid hormone plays a key role in many physiologic functions and is especially critical for brain and neurological development in the fetus and child, which highlights the public health importance of thiocyanate potentially affecting normal thyroid function (Braverman et al., 2005; Steinmaus, Miller, Cushing, Blount, & Smith, 2013; Tonacchera et al., 2004; Wyngaarden, Stanbury, & Rapp, 1953).

A high thiocyanate/iodine (SCN/I) ratio leads to a higher risk. In human studies, goiters due to the consumption of thiocyanate were more likely to occur when iodine levels were low, and were reversed with iodine supplementation (Ermans et al., 1983). Ayangade, Oyelola, and Oke (1982) found that in pregnant women the thiocyanate level of the cord blood was proportional to the maternal serum thiocyanate level, indicating that thiocyanate can cross the placental barrier and affect the fetus. According to the study of Laurberg, Nahr, Pedersen, and Fuglsang (2004), thiocyanate decreases breast milk iodine concentrations,
and may thus increase the risk of iodine deficiency-induced brain damage in the child. Besides, smoking mothers had significantly higher serum levels of thiocyanate, therefore, smoking during the period of breastfeeding exacerbate the harmful effects on thyroid function in breastfed infants. However, in a study in Boston-area where mothers and their breastfed infants are generally iodine sufficient, it showed that low levels of thiocyanate exposure do not affect infant serum thyroid function (Leung et al., 2012).

A low level of thiocyanate exposure is not likely to induce negative effects on thyroid function in healthy individuals. No significant effects on thyroid function (T4, T3, TSH) resulted from the consumption of 8 mg of thiocyanate in milk daily for 12 weeks, although serum and urinary thiocyanate levels increased (Dahlberg et al., 1984). The dietary contribution of milk borne thiocyanate to overall diet is also low compared to directly consuming thiocyanate rich foods such as brassica, which for example, contribute 14.7 mg/d as a national average in UK (Sones, Heaney, & Fenwick, 1984). The levels of thiocyanate expected from the augmentation of milk following codex could achieve 19 mg/L (data from this report). Feeding milk with 19 mg/L of thiocyanate was without effect on thyroid metabolism in one study with human subjects, at least over the month of the experiment (Dahlberg, Bergmark, Eltom, Björck, & Claesson, 1985). The clinically significant reduction in iodine uptake is likely to require a dose of 200–400 mg of thiocyanate, or the equivalent of 10–20 L of milk containing 20 mg/L of thiocyanate (Reiter & Härnulv, 1984), which underscores the risks of thiocyanate in milk are limited to situations where there has been an excessive addition of sodium thiocyanate.

Considering the potential for toxic impacts, sodium thiocyanate has been withdrawn as a permitted food preservative in milk products in China since 2007. In China, it has been also listed in the negative list of inedible substances possibly illegally added in food since 2008. At present, thiocyanate levels in milk products is regularly inspected by the food safety authorities in China. However, the natural occurrence of thiocyanate needs to be acknowledged. Thiocyanate is widely distributed in animal tissues and secretions, including the mammary, salivary and thyroid glands and in the stomach and kidneys (FSANZ, 2002). In human body fluids, thiocyanate levels typically range from 10 to 300 mg/L (Björck, Claesson, & Schulthess, 1979; Farrag & Marth, 1992; Reiter & Härnulv, 1984). In human milk, the range is 0.4–228 µg/L (Kirk, Dyke, Martin, & Dasgupta, 2007). In cow’s milk, the concentrations range from 1 to 35 mg/L (Fonteh, Grandison, & Lewis, 2002; Fweja, Lewis, & Grandison, 2008; Fweja et al., 2007; Ponce, 2005).

Thiocyanate in milk originates chiefly from cyanogenic glucosides and glucosinolates in plants used for cow feed. Cyanogenic glucosides are found in a wide range of plant species such as bitter almonds, linseed and cassava, and many pasture or feed species such as white clover and sorghum (Wood, 1975). When these plants are damaged by chewing, the cyanogenic glucosides are hydrolyzed by plant β-Glucosidase and α-Hydroxynitrile lyase to release hydrocyanic acid (HCN) (Gruhnert, Bielh, & Selmar, 1994). HCN is absorbed into the animal, and then detoxified by mammalian rhodanese (EC 2.8.1.1) and thiosulfate to form thiocyanate (Hall & Guest, 1992). Glucosinolates are found in crucifers such as cabbage, kale, brussel sprouts, cauliflower, turnips, rutabaga, and rape (Korhonen, 2004; Papas, Ingalls, & Campbell, 1979; Rosa, Heaney, Fenwick, & Portas, 1997). When plant tissue is damaged, glucosinolates may be hydrolyzed by plant thioglucoside glucosylhydrolase (myrosinase, EC 3.2.3.1), to release a range of compounds such as aglucones, nitriles, isothiocyanates, and thiocyanate (Van Etten & Wolff, 1973). The fate of most of the serum thiocyanate is excretion via the urine intact or as sulphate, but a small percentage equilibrates from serum into the milk (Wood, 1975).

Because of the risks associated with excess additions of thiocyanate to milk, it is important that levels of thiocyanate be controlled. But thiocyanate is also a naturally occurring substance in milk, so knowledge of the naturally-occurring presence of thiocyanate in raw milk is crucial to distinguish the source of thiocyanate detected in milk. The present study was designed to investigate variations in thiocyanate concentration and to aim at proposing a baseline concentration for naturally-occurring thiocyanate in raw milk, to serve as a reference concentration for the purposes of food safety supervision.

2. Materials and methods

2.1. Sampling

In 2013–2014, 2059 milk samples were collected, among which 1669 samples were collected from twelve provinces, including the most productive areas of milk, in China, 270 from New Zealand and 120 from the Netherlands. Among the samples, 860 samples were collected in summer and 862 in winter, and 1392 samples were collected from large-scale farms and 330 from small holdings. Samples were kept at low temperature and received by the laboratories within two days.

2.2. Quantification analysis and quality control

For analysis, samples were sent to a range of local laboratories, including the laboratory of National Food Quality Supervision and Inspection Center in China (24 Jiuxianqiao Middle Road, Chaoyang District, Beijing), seven laboratories of private dairy enterprises in China, two laboratories in New Zealand (AsureQuality Limited, 1c Quadrant Drive, Lower Hutt, Wellington and Analytica Laboratories, Ruakura Research Centre, Hamilton) and one in the Netherlands (TNO Triskelion, 3700 HE Zeist the Netherlands). Each laboratory used in house validated test methodology for the measurement of thiocyanate. In general, the aqueous phase was extracted from the milk samples by the addition of acetonitrile for protein precipitation, followed by defatting using a reverse-phase column filled with polydivinylbenezene polymer (or equivalent degreasing column, or commercialized RP column). Then appropriate aliquots of the samples and external standard solution (sodium thiocyanate) were injected separately into an ion chromatograph, with electric conductivity or UV detection. The anlyte identification was based on the retention time with respect to the standard peak, and the concentration of thiocyanate ion in test sample were obtained from the peak area ratio with respect to the external standard. The test method used at Analytica Laboratory included a stable isotope labeled internal standard, protein precipitation with acetonitrile, dilution and tandem mass spectrosdcopy detection.

For inter-laboratory performance evaluation prior to testing milk samples, standard samples of milk powder containing thiocyanate ion were measured by the seven laboratories of private dairy enterprises in China. The standard samples were also measured during each sample batch. In addition, 20% of the samples were duplicate-detected by two laboratories. As a performance requirement, the relative deviation of two outcomes of the same sample, calculated by the equation: relative deviation (%) = (A – B)/(A + B) × 100, should not exceed 20%.

2.3. Statistical analysis

All analyses were conducted using SAS 9.3 (Cary, NC). The Wilcoxon rank sum test and multivariable linear regression analysis were used to analyze the impacts of countries, seasons and pasture
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