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Milk protein composition and stability changes affected by iron in water sources

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ABSTRACT

Water makes up more than 80% of the total weight of milk. However, the influence of water chemistry on the milk proteome has not been extensively studied. The objective was to evaluate interaction of water-sourced iron (low, medium, and high levels) on milk proteome and implications on milk oxidative state and mineral content. Protein composition, oxidative stability, and mineral composition of milk were investigated under conditions of iron ingestion through bovine drinking water (infused) as well as direct iron addition to commercial milk in 2 studies. Four ruminally cannulated cows each received aqueous infusions (based on water consumption of 100 L) of 0, 2, 5, and 12.5 mg/L Fe^{2+} as ferrous lactate, resulting in doses of 0, 200, 500 or 1,250 mg of Fe/d, in a 4 × 4 Latin square design for a 14-d period. For comparison, ferrous sulfate solution was directly added into commercial retail milk at the same concentrations: control (0 mg of Fe/L), low (2 mg of Fe/L), medium (5 mg of Fe/L), and high (12.5 mg of Fe/L). Two-dimensional electrophoresis coupled with matrix-assisted laser desorption/ionization–tandem time-of-flight (MALDI-TOF/TOF) high-resolution tandem mass spectrometry analysis was applied to characterize milk protein composition. Oxidative stability of milk was evaluated by the thiobarbituric acid reactive substances (TBARS) assay for malondialdehyde, and mineral content was measured by inductively coupled plasma mass spectrometry. For milk from both abomasal infusion of ferrous lactate and direct addition of ferrous sulfate, an iron concentration as low as 2 mg of Fe/L was able to cause oxidative stress in dairy cattle and infused milk, respectively. Abomasal infusion affected both caseins and whey proteins in the milk, whereas direct addition mainly influenced caseins. Although abomasal iron infusion did not significantly affect oxidation state and mineral balance (except iron), it induced oxidized off-flavor and partial degradation of whey proteins. Direct iron addition to milk led to lipid oxidation during storage at 4°C. Oxidation level was positively associated with the concentration of added iron. Minerals (Mg, P, Na, K, Ca, Zn) in milk were not affected by the added iron in milk. This study indicated that a small amount of iron contamination in bovine drinking water at the farm or incidental iron addition from potable water sources causes oxidation, affects milk protein composition and stability, and affects final milk quality.
Key words: iron, protein, oxidation, milk synthesis

INTRODUCTION

Cow milk and related dairy products are nutritious foods containing numerous essential nutrients, especially milk proteins, which serve as an excellent source for essential amino acids. Milk proteins are composed of caseins (80%) and whey proteins (20%). Caseins are known to carry calcium and phosphate, which have many bioactive functions and contribute to efficient digestion (Haug et al., 2007). Whey proteins possess a variety of nutritional and biological properties and thus are widely used in reducing the risk of diseases such as cancer (de Wit, 1998; Gill and Cross, 2000), inflammation (Clare et al., 2003), chronic stress-induced disease (Ganjam et al., 1997), and human immunodeficiency virus infection (Oona et al., 1997; Micke et al., 2002). Bovine whey proteins perform their biological functions through constituents including β-LG (mediate and transport immunoglobulins during colostrum formation), α-LA (lactose synthase component and possible antimicrobial and anticancer activity), immunoglobulins (serving as antibodies to protect the mammary gland from infection), serum albumin (antimutagenic, anticancer, and immunomodulation activity), lactoferrin (iron-binding, iron transport, antimicrobial, antiinflammatory, and anticancer activities, immune system modulation), and lactoperoxidase (antimicrobial and

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antioxidant properties) (Swaisgood, 1995; Levieux and Ollier, 1999; Loimaranta et al., 1999; Haug et al., 2007; Adlerova et al., 2008; Alonso-Fauste et al., 2012).

Quality and yield of bovine milk proteins are determined by genetics of dairy cows, hormones, dietary energy, and lactation environment (Bionaz et al., 2012). Recently, excess iron ingestion was found to affect cow health through the formation of an oxidation environment and to interfere with absorption of other minerals (Hansen et al., 2010); this may contribute to increases in mastitis, bacterial infection, and retained fetal membranes, and a decrease in immunity (Standish et al., 1971; Bullen et al., 1978; Linn, 2008). A decline in the health status of dairy cattle immediately depresses their milk production and alters milk composition, including loss of milk yield (Gröhn et al., 2004), decrease in lactose and fat contents (Bansal et al., 2005), and increases in sodium, chloride, and electrical conductivity (Bruckmaier et al., 2004). However, effect of iron-induced oxidative stress on milk protein synthesis of lactating dairy cows has not been studied yet.

Dairy cattle drink 90 to 150 L of water each day when producing milk (Feng et al., 2013). A common resource for bovine drinking water is groundwater, where water can dissolve iron and other minerals as it percolates through soil and rock and hold the minerals in solution. The US Environmental Protection Agency (USEPA) reported that Fe concentration in ground water of many regions in the United States exceeds the secondary maximum contaminant levels (0.3 mg of Fe/L) set by that agency (USEPA, 2015). Due to the large amount of water consumed by dairy cows per day, ferrous Fe in milligrams-per-liter concentrations makes water a potentially significant source of iron intake for cows. In addition, although direct addition of water during fluid milk processing is not legal, incidental contamination may occur related to the use of potable water when cleaning equipment on the farm or in the processing plant. In addition, potable water is used as an ingredient in the manufacture of many dairy-based products, providing an avenue for the incidental addition of iron and contact with milk proteins. However, there is limited information about the effects of iron ingestion either in vivo or in vitro on quality of milk proteins.

This study is part of a larger interdisciplinary study involving the implications of water-sourced iron on milk quality. Feng et al. (2013) described the experimental conditions of the in vivo study on phosphorus absorption in lactating dairy cows. Concurrently, Mann et al. (2013) evaluated the subsequent effect of milk synthesized under the experimental conditions on fluid milk sensory quality. Our objective in this study was to evaluate the interaction of water-sourced iron (low, medium, and high levels), as determined through in vivo (water provided to dairy cattle) and in vitro (direct addition to whole processed milk) delivery, on the milk proteome and implications for other milk quality parameters, including oxidative state and mineral content. Our null hypothesis was that no differences in milk proteome, oxidative status, and mineral content would be observed using either the in vivo or the in vitro approach. Our alternative hypothesis was that both ways of iron ingestion would affect the milk proteome, affect milk mineral content, and contribute to changes in oxidative stability. Milk used for the in vivo portion of this study was sourced from this interdisciplinary study.

MATERIALS AND METHODS

Milk Samples Collected from Individual Farm Cows

Experiment Design and Raw Milk Collection. Protocols and procedures of this experiment were approved by the Virginia Tech Institutional Animal Care and Use Committee (12-027-DASC). Details of the in vivo experimental study are reported in Feng et al. (2013). In brief, 4 ruminally cannulated early lactation (second-lactation) cows (2 Holstein and 2 Holstein × Jersey cross) were isolated from external water sources to eliminate outside variables, housed in individual stalls, and placed on a standard basal diet and water source (Feng et al., 2013). Abomasal infusion of ferrous lactate was performed as described in our previous work (Feng et al., 2013). Treatments (abomasal infusion of 0, 200, 500, or 1,250 mg of Fe/d as ferrous lactate solution) were imposed in a 4 × 4 Latin square design with 14-d periods. Treatments were formulated to approximate 0, 2, 5, or 12.5 mg of Fe/L concentrations in drinking water, assuming water intake of 100 L/d. The infusion treatment design is summarized in Tables 1 and 2. Milk samples were collected from the evening milking of each cow at d 13 of each period, allowing for 6 d of total infusion time before milk collection, as described by Feng et al. (2013).

Milk Processing. Details of the milk processing are described in full in Mann et al. (2013). In brief, raw milk was collected in 5-gallon stainless steel milk cans and immediately transported to the Food Science and Technology Dairy Processing Laboratory, and stored in a cooler (Tonka, Hopkins, MN) at 4°C. Milk from each period for each cow was processed separately. After preheating to 55 to 60°C, milk was separated into cream and skim milk using a pilot-plant separator (Elecrem separator, model IG, 6400rpm, Bonanza Industries Inc., Calgary, AB, Canada). Cream was then added back to skim milk to achieve 3.18 ± 0.04% milk