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Characterization of the bovine milk proteome in early-lactation Holstein and Jersey breeds of dairy cows

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ABSTRACT

Milk is a highly nutritious natural product that provides not only a rich source of amino acids to the consumer but also hundreds of bioactive peptides and proteins known to elicit health-benefitting activities. We investigated the milk protein profile produced by Holstein and Jersey dairy cows maintained under the same diet, management and environmental conditions using proteomic approaches that optimize protein extraction and characterization of the low abundance proteins within the skim milk fraction of bovine milk. In total, 935 low abundance proteins were identified. Gene ontology classified all proteins identified into various cellular localization and function categories. A total of 43 low abundance proteins were differentially expressed between the two dairy breeds. Bioactive proteins involved in host-defense, including lactotransferrin ($P = 0.0026$) and complement C2 protein ($P = 0.0001$), were differentially expressed by the two breeds, whereas others such as osteopontin ($P = 0.1788$) and lactoperoxidase ($P = 0.2973$) were not. This work is the first to outline the protein profile produced by two important breeds of dairy cattle maintained under the same diet, environment and management conditions in order to observe likely true breed differences. This research now allows us to better understand and contrast further research examining the bovine proteome that includes these different breeds.

Biological significance: Within the last decade, the amount of research characterizing the bovine milk proteome has increased due to growing interest in the bioactive proteins that are present in milk. Proteomic analysis of low abundance whey proteins has mainly focused on human breast milk; however, previous research has highlighted the presence of bioactive proteins in bovine milk. Recent publications outlining the cross-reactivity of bovine bioactive proteins on human biological function highlight the need for further investigation into the bovine milk proteome. The rationale behind this study is to characterize and compare the low abundance protein profile in the skim milk fraction produced from Holstein and Jersey breeds of dairy cattle, which are two major dairy cattle breeds in the USA. A combination of fractionation strategies was used to efficiently enrich the low abundance proteins from bovine skim milk for proteomic profiling. A total of 935 low abundance proteins were identified and compared between the two bovine breeds. The results from this study provide insight into breed differences and similarities in the milk proteome profile produced by two breeds of dairy cattle.

1. Introduction

Milk is a valuable, natural product that provides a matrix of essential nutrients, growth factors and immune protection to offspring. Within the last five years, there has been a dramatic increase in the amount of published research focused on characterizing the milk proteome within different milk fractions, particularly in human milk [1–6]. Traditionally, milk proteins are categorized into three major groups: caseins, whey proteins and milk fat globule membrane (MFGM) proteins [6,7]. By using a wide range of fractionation techniques, whey proteins can be separated from caseins and further processed to allow extraction and identification of the low-abundance protein fraction in milk. Bioactive proteins and peptides contained in, or derived from, the whey fraction are involved in a wide range of physiological activities, including antioxidant activity, immuno-stimulating functions, anti-inflammatory effects, and protection against pathogen-induced intestinal inflammation [7–10]. Many of the milk bioactive proteins and peptides are also known to exhibit multifunctional physiological properties [11]. Thus, milk proteins are currently considered the most important source of bioactive peptides.

Cow's milk is an important nutrient for much of the human population, and studies have begun to characterize the bovine milk proteome, its bioactive profile, and the extent of cross-reactivity of bovine bioactive milk peptides on human biological function [12–14]. In bovine milk, the caseins (α_{S1}, α_{S2}, β and κ-casein) comprise approximately 80% of the total milk protein content, while whey proteins (primarily α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and serum albumin)

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represent the remainder. However, these highly abundant whey and casein proteins are far outnumbered by low-abundance proteins within the whey fraction. Reindhardt et al. [15] identified over 700 low-abundant whey proteins in skim bovine milk using quantitative proteomic techniques, including many with known immunological functions [15]. Lactoferrin is an important low-abundant protein involved in immune system development and is present in both human and bovine milk [16]. Positive health benefits from lactoferrin in human breast milk are well documented [7,8,17–19] and recent studies show similar responses when infants are fed formula supplemented with bovine lactoferrin [9,12,13,20,21]. Osteopontin, another bioactive protein present in both human and bovine milk, is recognized for its involvement in intestinal and immunological development in infants [8,22]. Despite there being only 63% amino acid similarity between bovine and human osteopontin, osteopontin in bovine milk exerts effects on human intestinal cell proliferation in vitro similar to osteopontin in human breast milk [8,22,23]. The cross-reactive nature of milk bioactive proteins provides opportunity to use bovine milk derived bioactive proteins as potential ingredients for health promoting foodstuffs and biopharmaceuticals.

As with human breast milk, many external and genetic factors influence the composition of bovine milk. A recent study characterized and compared the MFGM proteins within several different species including two bovine breeds, the Jersey and Holstein breeds of dairy cattle [24]. Using quantitative proteomic techniques, protein profiles were examined and principal component analysis scored the two breeds sharing similar proteomic patterns but also showing that each breed had distinctive MFGM proteins that were present at different concentrations. The Jersey MFGM contained a higher abundance of proteins with antimicrobial and angiogenic activities, whereas the Holstein MFGM contained proteins involved in immune system modulatory processes including antioxidant, anti-apoptotic, anticancer, and host cell protection activities [24]. Breed differences in alveolar dynamics [25], feed conversion efficiency [26,27], susceptibility to heat stress [28,29], and genetic variants existing for protein types [30] could have contributed to the observed differences in MFGM protein profile. However, additional factors known to affect milk composition in cattle, including diet, cow health, parity, environment, management practices and stage of lactation [31–33], could have also contributed to the observed breed differences. While genetics are estimated to contribute 55% of the variation observed in milk composition between breeds, the remaining 45% is explained by differing management factors [34]. Feeding different breeds of cows the same diet while being maintained in the same environment under the same management practices allows for a more direct comparison of true breed differences in the milk proteome. We hypothesize that when Holstein and Jersey breeds of dairy cattle are fed the same balanced diet and maintained under the same management and environmental conditions, there will be significant differences in the low abundance milk protein profile contained within the skim fraction of milk from the two breeds of dairy cows. The objective of this study is to characterize and differentiate the low abundance protein profile within the skim milk fraction produced by Holstein and Jersey dairy cows that are maintained under the same management practices and environmental conditions.

2. Materials and methods

2.1. Animals and diet

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont (Burlington, VT, USA). Six Jersey cows (80 ± 49 days in milk (DIM)) and six Holstein cows (75 ± 21 DIM) were housed in the same tie-stall barn at the Paul R. Miller Research and Educational Center (University of Vermont, Burlington, VT). All cows had free access to water and offered the same diet ad libitum (Table 1) for 7 days. All cows were fed individually for the duration of the trial. The diet consisted of a base forage ration that was fed twice daily (0600 and 1500 h) and a grain-based top-dress, which was thoroughly mixed into the offered forage three times a day at 0330, 1100, and 1800 h. Feed refusals from each animal were removed and weighed each morning and samples were stored at −20 °C until analysis. Refusal samples were subsequently dried at 65 °C for 48 h to calculate daily dry matter intake for each animal. Additional fresh feed and grain samples were collected and composited across the 7-d collection period for wet chemistry analysis (Dairy One, Ithaca, NY, USA).

Table 1
Ingredient and chemical composition of diets.

	Diet
Ingredient [61]	
Corn silage	36.6
Haylage	18.3
Soybean meal	7.4
Canola meal solvent	4.9
Citrus pulp dry	8.5
Amino max	7.3
Corn grain ground fine	10.8
Vitamin–mineral mix[a]	5.9
Nutrient composition	
DM[b] (%)	59.6
NDF[c] (% of DM)	35.7
CP[d] (% of DM)	18.7
NFC[e] (% of DM)	34.9

[a] Vitamin–mineral mix contained (DM basis): 5.5% PGI amino enhancer, 2.3% sodium sesquinate, 2.6% calcium carbonate, 1.3% salt, 1.4% PGI vitamin premix, 0.7% magnesium, 0.05% Zinpro 40, 0.02% Rumensin, 0.31% Diamune trace mineral.
[b] DM: dry matter.
[c] NDF: neutral detergent fiber.
[d] CP: crude protein.
[e] NFC: non-fiber carbohydrate.

2.2. Measurements and sampling

Cows were milked twice daily at 0400 and 1600 h. Milk yield was recorded at each milking and milk samples were collected during the morning and afternoon milking throughout the 7-day experiment (Tru-Test WB Ezi-Test Meters, DHIA, Lancaster, PA, USA). Milk samples for general milk component analysis were collected and preserved with bronopol and natamycin (D and F Control Systems, Inc., Broad Spectrum Microtabs, Norwood, MA, USA) and stored at 4 °C until commercial analysis (DHIA, Lancaster, PA, USA), which was performed within two days after collection. Milk subsamples collected for low abundance protein analysis were immediately frozen in a dry-ice ethanol bath after collection and stored at −80 °C until further analysis. Additional subsamples were chilled on ice immediately after collection and centrifuged at 4000 ×*g* for 10 min at 4 °C within 2 h of collection. The fat layer was removed and the skimmed milk samples were stored at −20 °C for high-abundance protein analysis.

Blood samples were collected by coccygeal venipuncture after milking (0800 and 1700 h) on day 0 and 7. Samples were collected into heparinized Vacutainers®(Becton Dickinson and Company, Franklin Lakes, NJ, USA) and stored on ice until centrifugation at 3000 ×*g* for 15 min at 4 °C for plasma collection. Plasma was transferred into polypropylene tubes and frozen at −20 °C until analysis. Commercially available kits were used to analyze to the plasma concentrations of β-hydroxybutyrate (BHBA; Sigma, Saint Louis, MO, USA), urea nitrogen (PUN; Teco Diagnostics, Anaheim, CA, USA), glucose (Sigma, Saint Louis, MO, USA), and non-esterified fatty acids (NEFA; ZenBio, Inc., Research Triangle Park, NC, USA). Samples were analyzed according to manufacturer's instructions and all coefficients of variance were <5%.