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Exploration of the bovine colostrum proteome and effects of heat treatment time on colostrum protein profile

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ABSTRACT

Heat treatment of colostrum is performed on modern dairy farms to reduce pathogenic contamination before hand-feeding the colostrum to newborn calves; however, limited data are available concerning effects of heat treatment on biologically active proteins in colostrum. The objective of this exploratory study was to investigate effects of heat treatment and length of heat treatment on colostrum protein profile. Colostrum samples were collected from Holstein cows within 12 h after parturition and assigned to the following groups: heat treatment at 60°C for 0 (untreated control), 30, 60, or 90 min. Samples were fractionated using acid precipitation, followed by ultracentrifugation and ProteoMiner (Bio-Rad Laboratories, Hercules, CA) treatment, and tandem-mass tagging was used to comparatively assess the low abundance protein profile. A total of 162 proteins were identified with more than 2 peptides in the low abundance protein enriched fraction. Of these, 62 differed in abundance by more than 2-fold in heat-treated samples compared with the unheated control. The majority of proteins affected by heat treatment were involved in immunity, enzyme function, and transport-related processes; affected proteins included lactadherin, chitinase-3-like protein 1, and complement component C9. These results provide a foundation for further research to determine optimum heat treatment practices to ensure newborn calves are fed colostrum-containing proteins with the highest nutritional and biological value.

Key words: low abundance milk protein, mass spectrometry, casein, whey

INTRODUCTION

Colostrum is a complex mixture of proteins, lipids, simple carbohydrates (primarily lactose), vitamins, and minerals. Several of these factors including immunoglobulins, growth factors, and cytokines can influence immune and gastrointestinal development following colostrum consumption in the first day of life (Ingram et al., 1956; Burrin et al., 1996; Yamanaka et al., 2003). In addition to these known compounds, recent data suggest that even small peptides present at low abundance in colostrum and milk may affect whole organ systems in the neonate. Data supporting this "lactocrine hypothesis" were reviewed by Bartol et al. (2013), detailing the mechanisms by which relaxin, a peptide secreted in sow colostrum and milk, can enhance development of the reproductive tract of female piglets. Such data indicate the vast potential of colostrum components for neonatal development.

On many dairy farms, newborn calves are hand-fed colostrum during the first 2 to 3 d after birth to ensure adequate consumption (Yang et al., 2015). One disadvantage of hand-feeding colostrum is increased risk of bacterial contamination of colostrum before feeding. Heat treatment of colostrum at 60°C for 60 min is becoming a common management tool to limit bacterial exposure in newborn calves (NAHMS, 2016). This protocol sufficiently reduced bacterial contamination, while minimally affecting IgG concentration and colostrum viscosity (Godden et al., 2006; Elizondo-Salazar et al., 2010). Little attention was paid to other potentially important colostrum proteins when heat treatment protocols were developed. A recent study that fed either unheated or heat-treated colostrum to calves observed reduced cytokine absorption in calves that received heat-treated colostrum, implying that heat treatment can affect proteins that would otherwise be absorbed and function in calf development (Gelsinger and Heinrichs, 2017).

Early work on the bovine colostrum proteome used centrifugation and ultracentrifugation to remove the fat

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layer and isolate whey from the casein pellet (Yamada et al., 2002; Palmer et al., 2006). Immunoabsorption and 2-D gel electrophoresis were used to separate proteins before microsequencing and tandem MS analysis. Differences in the 2-dimensional electrophoretic gel patterns were observed between colostrum and mature bovine milk. At that time, 29 low abundance proteins were identified as unique to colostrum (Yamada et al., 2002). Almost a decade later, an ion-exchange-based protein fractionation method was performed on bovine colostrum and fresh milk and identified over 140 uniquely colostral proteins (Le et al., 2011). Shortly thereafter, Nissen et al. (2012) identified over 400 proteins in bovine colostrum after fractionating colostrum samples into 8 different fluid and solid fractions using a range of fractionating techniques: ultracentrifugation, acid precipitation, and ultrafiltration. The most expansive analysis of the bovine colostrum proteome to date used ProteoMiner enrichment, affinity chromatography depletion, gel separation, and nano-HPLC-MS/MS techniques to identify over 600 proteins that occur in low abundance in colostrum (Altomare et al., 2016).

Based on gene ontology, the majority of proteins present in colostrum are involved in immune, enzymatic, and transport pathways (Zhang et al., 2015a), but the importance of each protein to neonatal calf development is yet to be determined. Additionally, no global investigation has been done to determine effects of heating on the colostrum proteome. Thus, the objective of this study was to compare the protein profile of colostrum before and after exposure to heat treatment for 30, 60, or 90 min. Durations of 30 and 60 min were chosen because they are currently used on dairy farms. A 90-min duration was chosen to provide an example and to represent any on-farm case when colostrum may be heated for a longer duration than recommended.

MATERIALS AND METHODS

Sample Collection and Heat Treatment

All animal procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (#46062). A detailed description of colostrum collection and preparation of unheated and 60-min heat treatment colostrum treatments is given by Gelsinger and Heinrichs (2017). Briefly, total first-milking colostrum was collected within 12 h after parturition from approximately 20 individual cows and stored at −20°C until treatment preparation. Approximately 114 L of colostrum was thawed at 4°C, pooled, and divided into 2 equal batches. One batch remained unheated and the other was heated to 60°C for 60 min (**heat60**) before returning to storage at −20°C as described by Gelsinger and Heinrichs (2017). The **heat30** and **heat90** treatments were created by heating 1-L aliquots of the unheated colostrum treatment to 60°C for 30 or 90 min, respectively. Aliquots were heated using a water bath and constant agitation. Colostrum and water temperatures were monitored constantly in both heating systems, and colostrum temperature never exceeded 60°C.

At the time of treatment preparation, 50 mL of whole colostrum was collected and submerged in a dry ice-ethanol bath immediately following heat treatment, or pooling in the case of unheated colostrum, and stored at −80°C for low abundance protein analysis. An additional 50 mL of colostrum was centrifuged at 4,000 × g at 4°C for 10 min. The skim milk fractions were isolated and stored at −20°C for high abundance protein analysis. All samples were packaged in dry ice and shipped to the University of Vermont (Burlington) for proteomics analysis.

High Abundance Protein Analysis

Skimmed colostrum samples (n = 1 sample/treatment) were thawed at 4°C overnight. Thawed samples were mixed thoroughly by vortexing, followed by sonication (Bransonic Model 220 sonicator, Branson Ultrasonics, Danbury, CT) at 33 W for 15 min in an ice water bath to maintain a sample temperature of less than 25°C. Following sonication, a 0.5-mL aliquot of milk was pipetted into a borosilicate test tube. Sample preparation and high abundance protein analysis were performed using methods outlined by Bordin et al. (2001) with modifications outlined by Tacoma et al. (2016). Briefly, 0.5 mL of reducing buffer (6.0 M guanidine hydrochloride, 5.0 mM trisodium citrate dehydrate, 20.0 mM dithiothreitol) was added to each sample, and samples were incubated at room temperature for 1 h, after which buffer without the dithiothreitol was added. The sample was transferred to a syringe and ejected through a 0.45-μm regenerated cellulose membrane syringe filter (Sartorius, Goettingen, Germany) into an HPLC autosampler vial. A Shimadzu (Kyoto, Japan) HPLC system fitted with a C_4 reversed-phase microbore analytical column (150 × 2.1 mm, 300 Å pore diameter, and 5 μm particle size, Vydac 214MS, Grace Davison Inc., Columbia, MD) maintained at 40°C was used for protein separation. Samples were maintained in the 15°C autosampler chamber before injection of 4 μL of sample into the HPLC. For protein separation, the following solvent protocol was applied: an increased linear gradient from 26.5 to 28% eluent B (90% acetonitrile and 0.1% trifluoroacetic acid in ultrapure water)