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Ratio of dietary rumen degradable protein to rumen undegradable protein affects nitrogen partitioning but does not affect the bovine milk proteome produced by mid-lactation Holstein dairy cows

R. Tacoma,* J. Fields,† D. B. Ebenstein,* Y.-W. Lam,†‡ and S. L. Greenwood*[1]
*Department of Animal and Veterinary Sciences,
†Vermont Genetics Network Proteomics Facility, and
‡Department of Biology, University of Vermont, Burlington, 05405

ABSTRACT

Little is known about the bovine milk proteome or whether it can be affected by diet. The objective of this study was to determine if the dietary rumen degradable protein (RDP):rumen undegradable protein (RUP) ratio could alter the bovine milk proteome. Six Holstein cows (parity: 2.5 ± 0.8) in mid lactation were blocked by days in milk (80 ± 43 d in milk) and milk yield (57.5 ± 6.0 kg) and randomly assigned to treatment groups. The experiment was conducted as a double-crossover design consisting of three 21-d periods. Within each period, treatment groups received diets with either (1) a high RDP:RUP ratio (RDP treatment: 62.4:37.6% of crude protein) or (2) a low RDP:RUP ratio (RUP treatment: 51.3:48.7% of crude protein). Both diets were isonitrogenous and isoenergetic (crude protein: 18.5%, net energy for lactation: 1.8 Mcal/kg of dry matter). To confirm N and energy status of cows, dry matter intake was determined daily, rumen fluid samples were collected for volatile fatty acid analysis, blood samples were collected for plasma glucose, β-hydroxybutyrate, urea nitrogen, and fatty acid analysis, and total 24-h urine and fecal samples were collected for N analysis. Milk samples were collected to determine the general milk composition and the protein profile. Milk samples collected for high-abundance protein analysis were subjected to HPLC analysis to determine the content of α-casein, β-casein, and κ-casein, as well as α-lactalbumin and β-lactoglobulin. Samples collected for low-abundance protein analysis were fractionated, enriched using ProteoMiner treatment, and separated using sodium dodecyl sulfate-PAGE. After excision and digestion, the peptides were analyzed using liquid chromatography (LC) tandem mass spectrometry (MS/MS). The LC-MS/MS data were analyzed using PROC GLIMMIX of SAS (version 9.4, SAS Institute Inc., Cary, NC) and adjusted using the MULTTEST procedure. All other parameters were analyzed using PROC MIXED of SAS. No treatment differences were observed in dry matter intake, milk yield, general milk composition, plasma parameters, or rumen volatile fatty acid concentrations, indicating no shift in total energy or protein available. Milk urea N and plasma urea N concentrations were higher in the RDP group, indicating some shift in N partitioning due to diet. A total of 595 milk proteins were identified, with 83% of these proteins known to be involved in cellular processes. Although none of the low-abundance proteins identified by LC-MS/MS were affected by diet, feeding a diet high in RUP decreased β-casein, κ-casein, and total milk casein concentration. Further investigations of the interactions between diet and the milk protein profile are needed to manipulate the milk proteome using diet.

Key words: milk protein, proteomics, bioactive, low-abundance protein

INTRODUCTION

It is well established that milk plays an important role in neonatal nutrition; however, research investigating the concept that milk proteins are a source of bioactive compounds that have physiological importance beyond AA provision is relatively sparse. Many of the identified bioactive peptides are released after cleavage of the high-abundance milk protein fraction, which include all casein isoforms, as well as whey proteins α-LA and β-LG. Identified low-abundance milk proteins, which include all other whey proteins, are known to have functionality as either cleaved peptide fragments or as entire intact proteins that can withstand gastric cleavage. These bioactive proteins and peptides, derived from both the low- and high-abundance protein fraction, have been identified to have a large breadth

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[1]Corresponding author: Sabrina.Greenwood@uvm.edu

of activity and play a role in human health, modulating physiological functions by various binding interactions with target cells and organs inducing beneficial physiological responses. Various functional properties associated with bioactive proteins and peptides include antimicrobial, antihypertensive, opioid, immunomodulatory, mineral binding, and antioxidative activities (Korhonen and Pihlanto, 2006; Sharma et al., 2011; Park and Nam, 2015). Investigation of human breast milk has identified several bioactive proteins and peptides that can influence infant health, particularly gut physiology and motility (Chatterton et al., 2013). Milk proteins present in bovine milk have also been identified to have bioactivity and cross-reactivity with human cells (Buccigrossi et al., 2007; Lönnerdal et al., 2011; Raikos and Dassios, 2014). Understanding secretion profiles of bovine milk proteins as well as mechanisms to manipulate this protein profile are important steps in further enhancing the healthfulness of bovine milk products.

The profile of proteins in bovine milk is influenced by animal factors such as breed, mastitis, and stage of lactation (Boehmer et al., 2010b; Zhang et al., 2015a; Tacoma et al., 2016). The mechanisms that affect the milk proteome at the cellular level within the mammary could be a result of AA supply or energy status on the transcriptional or translational efficiencies or rates within the cell (Osorio et al., 2016), or a result of posttranslational regulation such as changes in protein folding or intracellular protein transport (Ghazalpour et al., 2011). However, extracellular protein shifts may play just as important a role in determining the makeup of the milk proteome. Gene ontology (**GO**) analysis of the low-abundance bovine milk proteome fractions has consistently demonstrated that the majority of the identified proteins are extracellular in origin (Tacoma et al., 2016; Wang et al., 2017), suggesting that not only cell-specific regulation within the mammary gland as outlined above could affect the milk proteome, but that nonsecretory cell specific metabolic changes could also lead to shifts in the milk proteome via junction leakage, or para- or trans-cellular passage of proteins.

Altering the milk protein profile and bioactive properties of the milk by manipulating the diet of the dairy cow offers a promising approach to naturally enhance the healthfulness of milk products. Research examining the relationship between nutrition and the bovine milk protein profile is limited and nutrition is a significant management factor that has the potential to alter milk protein composition (Kennelly et. al., 2005; Tripathi, 2014). Christian et al. (1999) altered the proportions of high abundance bovine milk proteins by feeding a lupin-wheat-based diet, a high RUP source, to lactating dairy cows compared with cows fed spring-pasture, a high RDP source. Cows offered the lupin-wheat-based diet had higher concentrations of α_{S1}-CN, α_{S2}-CN, and γ-CN in the milk compared with cows on the high pasture diet, whereas concentrations of β-CN and κ-CN were present at higher concentrations in milk from cows fed spring pasture compared with cows on the lupin-based diet. More recently, a study was published outlining changes in high abundance milk protein expression patterns in response to inclusion of different corn and soybean feedstuffs in the ration. Although the type of corn included in the diet did not influence the milk protein profile, inclusion of heat-treated soybean meal resulted in a decrease in β-CN and zinc-α-2-glycoprotein fragments indicating the availability of RDP to influence secretion of specific milk proteins. These authors also reported differential expression of α-LA and zinc-α-2-glycoprotein due to diet, suggesting that ruminal microbial protein synthesis could affect the milk protein profile (Li et al., 2015). Mechanistically, shifts in the MCP versus diet-derived digestible RUP fractions reaching the small intestine are known to alter postabsorptive N metabolism, particularly affecting intestinal, hepatic, renal, and muscular metabolism (Hristov et al., 2004; Reynal and Broderick, 2005; Brito and Broderick, 2007), and would alter the blood proteome and N available for uptake and use for mammary protein synthesis but also the profile of nonmammary derived extracellular proteins within the milk via the mechanisms described above. Upon closer investigation of diets used in the research outlined by Christian et al. (1999) and Li et al. (2015), it is clear that they include diets with different RDP:RUP ratios; however, other nutrient differences between diets have made the interpretation of the effect of dietary protein content on the milk proteome difficult.

We hypothesize that it is the difference in diet RDP:RUP protein fraction that ultimately leads to a change in the bovine milk proteome. The goal of our study was to create 2 isonitrogenous and isoenergetic dairy rations with at least a 10% difference in the RDP:RUP ratio and examine the bovine milk proteome in milk samples collected from cows consuming these different diets.

MATERIALS AND METHODS

Experimental Design

Six mid-lactation Holstein dairy cows (parity: 2.5 $\pm$ 0.8) were blocked by DIM (80 $\pm$ 43 DIM) and milk yield (57.5 $\pm$ 6.0 kg) and then were randomly divided into 2 experimental groups in a double-crossover de-