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Colostrum protein uptake in neonatal lambs examined by descriptive and quantitative liquid chromatography-tandem mass spectrometry

Lorenzo E. Hernández-Castellano,*† Anastasio Argüello,* André M. Almeida,‡§ Noemí Castro,*[1] and Emøke Bendixen#

*Department of Animal Science, Universidad de Las Palmas de Gran Canaria, 35413 Arucas, Gran Canaria, Spain
†Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland
‡Instituto de Investigação Científica Tropical (IICT) and Centro Interdisciplinar de Investigação em Sanidade Animal (CIISA), 1300-142 Lisboa, Portugal
§Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa (ITQB/UNL), 2780-157 Oeiras, Portugal
#Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus, Denmark

ABSTRACT

Colostrum intake is a key factor for newborn ruminant survival because the placenta does not allow the transfer of immune components. Therefore, newborn ruminants depend entirely on passive immunity transfer from the mother to the neonate, through the suckling of colostrum. Understanding the importance of specific colostrum proteins has gained significant attention in recent years. However, proteomics studies of sheep colostrum and their uptake in neonate lambs has not yet been presented. The aim of this study was to describe the proteomes of sheep colostrum and lamb blood plasma, using sodium dodecyl sulfate-PAGE for protein separation and in-gel digestion, followed by liquid chromatography-tandem mass spectrometry of resulting tryptic peptides for protein identification. An isobaric tag for relative and absolute quantitation (iTRAQ)-based proteomics approach was subsequently used to provide relative quantification of how neonatal plasma protein concentrations change as an effect of colostrum intake. The results of this study describe the presence of 70 proteins in the ovine colostrum proteome. Furthermore, colostrum intake resulted in an increase of 8 proteins with important immune functions in the blood plasma of lambs. Further proteomic studies will be necessary, particularly using the selected reaction monitoring approach, to describe in detail the role of specific colostrum proteins for immune transfer to the neonate.

Key words: colostrum, immunity, proteomics, isobaric tag for relative and absolute quantitation (iTRAQ)

INTRODUCTION

It is well known that early colostrum intake is a key factor for neonatal survival in mammals. In ruminants, the complexity of the synepitheliochorial placenta does not allow sufficient transfer of immunoglobulins from the dam to the fetus (Argüello et al., 2004a; Castro et al., 2005, 2011). Consequently, lambs are classified as hypo-gammaglobulinemic at birth, and are entirely dependent on passive immunity transfer (**PIT**) from the mother to the neonate through early suckling of colostrum (Stelwagen et al., 2009; Danielsen et al., 2011). Colostrum contains essential nutrients such as fat, lactose, vitamins, and minerals (Ontsouka et al., 2003; Hernández-Castellano et al., 2014a; Lérias et al., 2014), but it is also rich in unique proteins that play active roles in regulating growth and development of the gut tissue. Colostrum also protects the neonate against pathogens and postpartum environmental challenges (Bendixen et al., 2011; Hernández-Castellano et al., 2015).

Although the transfer of immunoglobulins is a key factor in host defense to pathogens, a wider range of colostrum components have been suggested to contribute to the early protection of the neonate (Smith and Foster, 2007). These include many unique proteins with specific functional roles, including proteins secreted by neutrophils and macrophages, blood complement system proteins, acute-phase proteins, and specific proteins and peptides that have direct antimicrobial activity (Rainard and Riollet, 2006; Oviedo-Boyso et al., 2007; Leitner et al., 2008). In addition to mediating host defense, a wide range of bioactive colostrum proteins also play important roles as key regulators of gastrointestinal growth and development in early life (Bendixen et al., 2011).

Colostrum proteins are protected by trypsin inhibitors present in colostrum (Ramos et al., 2010; Hernán-

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[1]Corresponding author: noemi.castro@ulpgc.es

dez-Castellano et al., 2014a), as well as by low proteolytic activity in the gastrointestinal tract of newborn animals (Guilloteau et al., 1983). Additionally, the uptake of entire proteins through the gut epithelium is facilitated by unique features of a regulated apoptotic process of the neonatal ruminant enterocytes, which allows unrestricted passage of large molecules, such as immunoglobulins, into the gut (Castro-Alonso et al., 2008; Stelwagen et al., 2009). It is crucial to feed newborn ruminants colostrum during the first 48 h after birth, because the mechanisms that facilitate PIT and colostrum uptake are only functional in the first days of life (Moore et al., 2005; Hernández-Castellano et al., 2014b). Neither the cellular mechanisms of colostrum protein transfer to blood nor their bioactive roles in the neonate ruminants are fully understood. Although bovine milk and colostrum proteomes have attracted much attention, including reports of more than 253, 138, and 403 proteins characterized in colostrum and milk whey (Le et al., 2011), milk fat globule membrane (Reinhardt and Lippolis, 2008), and colostrum samples (Reinhardt and Lippolis, 2008; Le et al., 2011; Nissen et al., 2012), respectively, very limited knowledge seems to be available on sheep colostrum proteins. Some ELISA-based studies have reported concentration levels of IgG in sheep colostrum to range from 48.1 to 60.9 mg/mL (Moretti et al., 2010a,b; Higaki et al., 2013) and in newborn lamb plasma from 13.7 to 26.6 mg/mL (Quigley et al., 2002; Rodinova et al., 2008). However, proteomic studies of sheep colostrum proteins and their uptake in neonate lambs has not yet been performed.

The aim of this study was to describe the proteomes of sheep colostrum and lamb blood plasma, using an SDS-PAGE for protein separation and in-gel digestion, followed by liquid chromatography-tandem mass spectrometry (**LC-MS/MS**) of resulting tryptic peptides for protein identification. An isobaric tag for relative and absolute quantitation (**iTRAQ**)-based proteomics approach was subsequently used to provide relative quantification of changes in neonatal plasma protein concentrations as an effect of early colostrum intake.

MATERIALS AND METHODS

Animal Experimentation Disclosure

This experiment was approved by the ethics committee of the Universidad de Las Palmas de Gran Canaria (Spain) in compliance with animal experimentation regulations in the Canary Islands, Spain, and the European Union. A. M. Almeida holds a Federation of Laboratory Animal Science Associations (FELASA) grade C license, enabling him to design and conduct animal experiments in the European Union.

Sample Collection

The study was based on individual analysis of plasma samples from 8 single partum lambs (Canarian dairy breed), studied as 2 experimental groups (4 lambs each), as well as 2 samples from the pool of colostrum used for lamb feeding. As dams were estrous synchronized and subsequently mated, all lambs were born in the same period (May 2012) with a difference of a few days. The experiment took place at the experimental farm of the Veterinary Faculty of the Universidad de Las Palmas de Gran Canaria (28°8′20.66″ N, 15°30′24.97″ W, Gran Canaria, Spain). During the experimental period (from birth until 14 h after birth), the colostrum (**C**) group received colostrum feeding at 2 h after birth; the no-colostrum (**NC**) group was not fed at 2 h after birth. To ensure survival of the animals, both groups were subsequently fed with colostrum after the experimental period at 14 and 26 h after birth. Animals were fed a common pool of sheep colostrum with an IgG concentration of 64.74 mg/mL. Blood samples were collected directly before feeding at 2 and 14 h after birth from the jugular vein in 2.5-mL tubes with K-EDTA. Blood was centrifuged at 2,190 × *g* for 5 min at 4°C (Hettich-Zentrifugen, Universal 32 R, Tuttlingen, Germany) and the obtained plasma was frozen at −80°C until further analysis. All lambs were housed in rearing rooms with at least 0.3 m^2 of floor space per lamb. Each room had central heating with a room temperature of approximately 20°C.

Sample Preparation

Plasma and colostrum samples were analyzed at Aarhus University (Aarhus, Denmark). A total of 200 μL from each sample were homogenized with 1 mL of Tris-EDTA-sucrose (**TES**) buffer (10 m*M* Tris-HCl, pH 7.6, 1 m*M* EDTA, 0.25 *M* sucrose) using an UltraTurrax homogenizer (T10 basic, IKA-Werke, Staufen, Germany) at 12,000 rpm. Homogenates were centrifuged at 10,000 × *g* for 30 min at 4°C to remove insoluble components. Protein concentration of the supernatant was determined with the Quick Start Bradford Protein Assay (Bio-Rad, Hercules, CA), using BSA as standard reference (Bradford, 1976) and following the manufacturer's instructions. Aliquots of 100 μg of protein from each sample were recovered after precipitation with 6 volumes of ice-cold acetone (−20°C) and centrifugation at 15,000 × *g* for 10 min at 4°C.

For the descriptive analyses of plasma and colostrum proteomes, 60 μg of protein from the experimental groups (C and NC groups) at 14 h (2 biological replicates from each group) as well as the pooled colostrum used for the feeding experiment were prepared (2 technical replicates). Aliquots were resuspended in 20 μL of TES buffer. The