J. Dairy Sci. 100:1–11
https://doi.org/10.3168/jds.2017-12557


# Two-dimensional gas chromatography time-of-flight mass spectrometry-based serum metabolic fingerprints of neonatal calves before and after first colostrum ingestion

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## ABSTRACT

Neonatal calves show a remarkable increase in serum IgG levels after first ingestion of colostrum. They can absorb high-molecular IgG from colostrum in the small intestine by nonspecific receptor-independent fluid pinocytosis within 24 h after birth. However, little is known about the temporal changes in serum small-molecule metabolites, such as carbohydrates and AA, in neonatal calves after first colostrum ingestion. In this study, we examined temporal changes in serum metabolites of neonatal calves after first ingestion of colostrum by comprehensive 2-dimensional gas chromatography mass spectrometry (GCxGC-MS). Forty serum samples obtained from 5 calves at 8 time points between 0 and 12 h after first colostrum ingestion were analyzed in triplicate by GCxGC-MS. Multivariate analyses of 120 GCxGC-MS results revealed significant variations in the serum metabolites, primary individual differences among the calves, and secondary temporal changes within each individual calf. Several serum metabolites increased temporally after ingestion in each calf, but only a limited number of compounds were increased universally in all 5 calves. Eight compounds, including oligosaccharides such as lactose, were associated with temporal changes in IgG. Some essential AA that must be supplied from the diet increased temporally after ingestion, but differed from the temporal pattern of the oligosaccharides and IgG. These results suggest that the colostral contents may be absorbed by complex mechanisms that include intestinal pinocytosis for IgG and oligosaccharides, along with others such as specific transporters in the intestinal epithelial cells for AA in calves.

**Key words:** calf, colostrum, serum metabolomics, GCxGC-MS

## INTRODUCTION

It is important to feed calves adequate amounts of colostrum within 24 h after birth for dairy management because colostrum is the only source of nutrients in calves during the neonatal period. It also supplies immunity substances such as IgG to calves that are born without maternal transmission of IgG (Weaver et al., 2000; Chigerwe et al., 2015; MacFarlane et al., 2015). Therefore, neonatal calves that consume a sufficient amount of colostrum may grow healthy and be immune to infectious diseases that cause diarrhea and pneumonia (Furman-Fratczak et al., 2011; Raboisson et al., 2016).

The digestive tracts of mature mammals break down food contents into small molecules and then absorb them into cells via specific transporters (Chen et al., 2016; Glatz and Luiken, 2016; Satsu, 2017). For example, proteins are degraded into small peptides and AA that are absorbed by peptide and AA transporters, respectively, in the small intestine (Daniel, 2004; Bröer, 2008; Poncet and Taylor, 2013). In contrast, most neonatal mammals, including calves, can absorb colostral macromolecules such as proteins in the epithelial cells of the small intestinal brush border within a short time after birth (Staley et al., 1972; Staley and Bush, 1985; Jochims et al., 1994; Kaup et al., 1996; Moussaoui et al., 2014). It is well known that the serum concentrations of bioactive IgG are markedly increased after first colostrum ingestion (Quigley et al., 2002; Mokhber-Dezfooli et al., 2012; Osaka et al., 2014; Elsohaby and Keefe, 2015; Yang et al., 2015). Recent studies proposed that nonspecific receptor-independent fluid pinocytosis absorb colostral IgG in the small intestine of neonates (Sockolosky and Szoka, 2015; Martins et al., 2016). Based on these previous reports, we hypothesized that colostral small molecule compounds are also absorbed by the fluid pinocytosis and transferred into the blood, resulting in a significant variation in serum levels of such compounds and their metabolites before and after first ingestion of the colostrum in calves, as is the case

Received January 5, 2017.
Accepted January 29, 2017.
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for IgG. However, surprisingly little is known about the temporal changes in serum small molecule metabolites, such as carbohydrates, AA, and lipids, in neonatal calves after first colostrum ingestion, except for some blood biochemical parameters such as glucose, BUN, nonesterified fatty acids, and triglycerides (Hammon et al., 2002; Muri et al., 2005; Blum, 2006). To test our hypothesis, we first performed a comprehensive analysis of serum metabolites before and after first colostrum ingestion in neonatal calves.

Metabolomics is a powerful method used to comprehensively profile serum metabolites (Psychogios et al., 2011; Zhang et al., 2012; Mussap et al., 2013). Gas chromatography-mass spectrometry is commonly used in metabolomics studies (Begley et al., 2009; Sun et al., 2015; Wang et al., 2016). In conventional GC-MS, because metabolites in samples are separated on a GC capillary column, peak capacity limitations, such as co-elution of several metabolites at the same retention time, often become problematic in samples with complex chemical profiles. Two-dimensional gas chromatography (**GCxGC**)-MS overcomes this problem by using 2 different polarity columns (i.e., nonpolar and polar columns; Almstetter et al., 2012; Weinert et al., 2015). Two different separation processes in GCxGC increase the separation, detection, and identification of a wide variety of metabolic compounds relative to one-dimensional GC-MS (Zhang et al., 2012; Winnike et al., 2015). In GCxGC, effluents from the first-dimension (**1D**) column are trapped in a temperature-controlled modulator for a couple of seconds and then injected into the second-dimension (**2D**) column. During the process, compounds still co-eluting at the end of the 1D separation due to similar boiling points can be separated based on their different polarities in the 2D phase. As a result, each compound is characterized by both 1D and 2D retention times; thus, GCxGC-MS can detect hundreds to thousands more compounds than conventional GC-MS.

In the present study, we performed GCxGC-MS-based serum metabolomics in neonatal calves before and after first ingestion of colostrum. Multivariate analyses of GCxGC-MS results characterized individual and temporal variations in calf serum metabolic profiles. These findings will give new insights to understand the metabolism and absorption processes of colostral contents in neonatal calves.

## MATERIALS AND METHODS

### Blood Sampling from Neonatal Calves

Five male Holstein-Friesian calves with a mean BW at birth of 47.4 ± 3.2 kg were used in this experiment. This study was approved by the Animal Research Committee and followed the Animal Experiment Guidelines of Iwate University. The physical condition of each calf was evaluated based on appearance, vitality, and blood cell counts. Approximately 20 L of pooled colostrum was prepared from 12 Holstein-Friesian dams. The pooled colostrum was stored at −20°C until use. Protein and fat contents in the colostrum were 15.8 and 5.5%, respectively. The IgG concentration of the pooled colostrum was 56.6 g/L. Calves were fed the colostrum one time at a volume of 4% of BW using a rubber nipple attached to a bucket within 4.2 ± 0.4 h after birth. Blood samples were obtained from the jugular vein at 8 time points: before colostrum ingestion (0 h) and at 1, 2, 3, 4, 6, 8, and 12 h after ingestion. Serum samples were stored at −30°C until further analyses. Serum IgG concentrations were measured using a commercially available bovine IgG ELISA quantitation kit (Bethyl Laboratory Inc., Montgomery, TX).

### Sample Preparation

The GC-MS samples were prepared according to the previous procedures (Dunn et al., 2011). In brief, each serum sample was thawed on ice for 60 min, and an aliquot (100 μL) was transferred into a glass tube containing 400 μL of acetone with propanedioic acid (1 μg) as an internal standard. After mixing the samples thoroughly, the sample was centrifuged for deproteinization at room temperature at 560 × *g* for 10 min at 20°C. The supernatant was then transferred to a new glass tube and dried using a centrifugal vacuum evaporator until completely desiccated without heating. Each dried sample was derivatized using a 2-step method. First, 50 μL of fresh *O*-methylhydroxylamine in pyridine (20 mg/mL) was added to the sample, and the mixture was incubated at 80°C for 15 min. Next, 50 μL *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide was added to the sample and the mixture was incubated for trimethylsilyl (**TMS**) derivatization at 80°C for 15 min. Each sample was analyzed by GCxGC-MS within 24 h after TMS derivatization.

### GCxGC-MS Analysis

All samples were analyzed on a system containing an Agilent 7890 GC (Agilent Technologies, Santa Clara, CA) with a Gestel MPS2 autosampler (Gestel, Mülheim an der Ruhr, Germany) interfaced with a LECO Pegasus 4D time-of-flight MS (LECO Corp., St. Joseph, MI). The primary column, 30 m DB-5MS (0.25 mm i.d. × 0.25 μm df, Agilent Technologies), and the secondary column, 1.5 m RTX-200 (0.18 mm i.d. × 0.25 μm df, Restec Corp., Bellefonte, PA), were used for GCxGC.