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Changes in serum metabolites in response to ingested colostrum and milk in neonatal calves, measured by nuclear magnetic resonance-based metabolomics analysis

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ABSTRACT

Uptake of colostrum is of central importance for establishing a passive immunity transfer in neonatal calves. Studies of absorption and transmission of colostral immunoglobulins have been widely reported; however, changes in the serum in response to the absorption of colostral components in neonatal calves have not been completely characterized. Here, a nuclear magnetic resonance-based metabolomics approach was used to investigate the changes in metabolites in ingested colostrum, milk, and serum after neonatal calves were fed colostrum or milk. Twenty-seven neonatal male Holstein calves were assigned to 1 of the following groups: (1) calves not fed colostrum or milk and slaughtered approximately 2 h after birth (control group, n = 6), (2) calves fed colostrum at 1 to 2 h after birth and slaughtered 8 h after birth (n = 6), (3) calves fed 2 colostrum meals (at 1–2 and 10–12 h after birth) and slaughtered 24 h after birth (n = 6), (4) calves fed 3 colostrum meals (at 1–2, 10–12, and 22–24 h after birth) and slaughtered 36 h after birth (n = 6), or (5) calves fed 2 milk meals (1–2 and 10–12 h after birth) and slaughtered 24 h after birth (n = 3). Concentrations of valine, leucine, lactate, lysine, and isoleucine were higher and concentrations of lactose were lower in the groups fed colostrum and milk compared with groups not fed colostrum and milk, respectively. Metabolite changes between groups fed or not fed colostrum and milk were similar and may reflect the primary metabolic requirements of ingestion by the small intestine of neonatal calves. Concentrations of serum metabolites choline, valine, leucine, and glutamate were higher in the serum of calves that received colostrum compared with control calves. Furthermore, concentrations of serum phenylalanine, valine, and glutamate were significantly higher, whereas serum concentrations of citrate and very low density lipoproteins were lower in calves that received colostrum compared with calves fed milk. Our results indicate that concentrations of leucine, valine, and glutamate, which were higher in the calves that ingested colostrum, may transfer into the bloodstream, and that these metabolites are associated with health benefits in the neonatal calves that received colostrum. These findings provide novel information to help us understand the mechanism by which colostrum components are metabolized and absorbed in the small intestine and then transferred into bloodstream of neonatal calves.
Key words: serum, colostrum, intestine, metabolomics

INTRODUCTION

Colostrum contains many bioactive and nutritional components and is a unique diet for neonates. Most importantly, colostral immunoglobulins play a central role in neonatal survival, as transmission into the bloodstream establishes a passive immunity transfer within the first 24 h of life (Quigley, 2004; Osaka et al., 2014). For this reason, many studies have investigated colostral and blood immunoglobulins and the relationship between them (Stott et al., 1979a,b; Osaka et al., 2014). In addition to establishing a passive immunity transfer, colostrum intake plays a role in stimulating intestinal mucosal growth and function as well as in enhancing the absorptive capacity of the gastrointestinal tract (Hammon et al., 2013; Steinhoff-Wagner et al., 2014). As a result, studies have found that glucose and galactose originating from lactose and xylose are absorbed in the small intestine and transferred into the bloodstream (Girard, 1990; Scharrer and Grenacher, 2000; Steinhoff-Wagner et al., 2011a). However, increases in concentrations of several growth factors, such as IGF-I and IGF-II, and insulin in the blood of calves depended on the timing of first colostrum feeding (Hammon et al., 2000), whereas absorption of colostral growth factors was barely observed in the intestine (Hammon and Blum, 1997; Ontsouka et al., 2004a). These results suggest that colostrum feeding in neonates does not only provide abundant bioactive substances and nutrition, but also contributes to improving the intestinal function for the absorption of specific colostral components.

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Although colostrum digested in the small intestine of neonatal calves has received little attention, studies on changes in the serum of neonatal calves have been widely reported. In previous studies, increases in concentrations of several free EAA and decreases in glutamine concentrations were observed in serum after calves received colostrum (Hammon and Blum, 1999; Zanker et al., 2000). Additionally, concentrations of essential fatty acids, carotene, retinol, and α-tocopherol were significantly higher in serum of calves fed colostrum on d 1 postcalving compared with delayed colostrum-fed calves (Blum et al., 1997). These results provide useful information for understanding the effects of colostrum intake on blood components and the potential health benefits for neonatal calves. It is notable that colostrum is a significant source of metabolites, as several metabolites can be used as potential bioactive compounds (Sundekilde et al., 2013). However, changes in blood metabolites related to colostrum intake have not been fully elucidated in neonatal ruminants.

Metabolomics, an omics field in systems biology, is widely used to investigate small metabolites within biological samples (Putri et al., 2013). As nuclear magnetic resonance (**NMR**) spectroscopy is a powerful method that provides direct relationships between metabolite contents and resonances, NMR approaches have been widely used to investigate the changes in metabolites of milk or serum, as well as correlations between milk and serum metabolites in dairy animals in recent years (Sundekilde et al., 2011; Klein et al., 2012; Palma et al., 2016). The results of such studies indicate that NMR-based metabolomics techniques provide a powerful tool for exploration of the effects of colostrum intake on serum metabolites in neonatal calves.

The objective of our study was to use an NMR approach to investigate the changes in serum metabolites in response to the absorption of colostrum or milk in the small intestine of neonatal calves, and to explore the absorption of metabolites into the bloodstream. We hypothesized that colostrum could be absorbed in the gastrointestinal tract and that a wide range of small molecules would transfer into the bloodstream of the calves fed colostrum. We expected our findings would provide novel information for a better understanding of the changes in serum metabolites corresponding to colostrum uptake and digestion in the gastrointestinal tract.

MATERIALS AND METHODS

Animals and Experimental Procedures

Twenty-seven neonatal male Chinese Holstein calves from the Chuzhou dairy farm (Chuzhou, China), with birth weight of 40 ± 2 kg (±SD), were used in this study (Supplemental Figure S1; https://doi.org/10.3168/jds.2017-14287). Animal care and use procedures were approved by the Animal Care Advisory Committee of the Anhui Academy of Agricultural Sciences. After birth, calves were separated from their mothers to prevent them from directly suckling colostrum. The first 2 milking colostrum samples were taken from healthy, multiparous Holstein dairy cows and pooled, and bulk milk from a healthy herd of experimental dairy farm cows was collected, placed in plastic bottles, and then stored at −20°C. Before use, the colostrum (not ingested colostrum) or milk (not ingested milk) was incubated in a water bath at 40°C and provided at approximately 8.0% of BW for each calf via a stomach tube, as described in previous studies (Chigerwe et al., 2012; Conneely et al., 2014). Six calves were not fed colostrum or milk and were slaughtered approximately 2 h after birth. These 6 calves constituted the control (**Ct**) group. Six calves received 1 colostrum meal at 1 to 2 h after birth and were slaughtered approximately 8 h after birth (**CI** group). Six calves received 2 colostrum meals at 1 to 2 and 10 to 12 h after birth and were slaughtered approximately 24 h after birth (**CII** group). Six calves received 3 colostrum meals at 1 to 2, 10 to 12, and 22 to 24 h postpartum and were slaughtered approximately 36 h after birth (**CIII** group). Three calves were fed milk instead of colostrum after birth, with 2 milk meals at 1 to 2 and 10 to 12 h after birth; milk-fed calves were slaughtered approximately 24 h after birth (**M** group). Samples of colostrum and milk were frozen and transferred to the laboratory.

Blood samples were collected from the jugular vein of individual calf in all groups before the calves were slaughtered. Samples were stored overnight at room temperature and centrifuged at 3,000 × g for 15 min at 4°C. Supernatant was collected and aliquoted into 1.5-mL tubes, then frozen at −20°C. After the blood samples were collected, the calves were slaughtered. Mid-duodenum, -jejunum, and -ileum segments were separated within 30 min after opening the abdominal cavity (Liang et al., 2014). Ingested colostrum or milk was collected from mid-jejunum segments, frozen in liquid nitrogen, and then stored at −80°C until analysis.

NMR Spectroscopy Analysis

Colostrum and milk samples were thawed at room temperature and centrifuged at 4,000 × g for 30 min at 4°C. The fat layer was removed and skim milk was collected. A total of 500 μL of skim milk from each sample was mixed with 1.0 mL of methanol and 500 μL of double-distilled H_2O, followed by periodical vortexing. The mixture was centrifuged at 12,000 × g for 10