¹H nuclear magnetic resonance-based plasma metabolomics provides another perspective of response mechanisms of newborn calves upon the first colostrum feeding¹

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ABSTRACT: The first meal of a neonatal calf after birth is crucial for survival and health. Blood IgG levels remarkably increase in neonatal calves after the first colostrum feeding. However, there is little comprehensive information on blood small-molecule metabolites in neonatal calves at that time. In this study, the changes in plasma metabolites of neonatal calves after the initial colostrum feeding were first examined with comprehensive ¹H nuclear magnetic resonance (NMR). Sixteen plasma samples obtained from 8 calves before and after feeding were analyzed with ¹H NMR. Multivariate analyses revealed a significant difference in metabolic profiles. After feeding, acute phase N-acetylated glycoproteins and

13 other plasma metabolites decreased, whereas 19 plasma metabolites increased. Metabolomics pathway analysis of these metabolites revealed that a global metabolic response on the first colostrum feeding was reflected by alterations of 13 metabolic pathways including lipid, carbohydrate, and amino acid metabolism in neonatal calves. These results suggested that besides meeting energy demand, a 4.0 L of high-quality colostrum feeding within 4 h after birth had a positive effect on relieving the postnatal stress in neonatal calves. This study provides another perspective of response mechanisms of newborn calves upon the first colostrum feeding.

Key words: ¹H nuclear magnetic resonance, metabolomics, metabolomics pathway analysis, colostrum, newborn calves

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INTRODUCTION

The timely feeding of an adequate volume of high-quality colostrum immediately after birth is one of the key factors influencing the health and survival of neonatal dairy calves (Godden, 2008; Langel et al., 2015; Yang et al., 2015). Colostrum intake provides high amounts of fats, proteins, peptides, vitamins, enzymes, hormones, growth factors, cytokines, minerals, and nucleotides

(Steinhoff-Wagner et al., 2014). Colostrum feeding stimulates intestinal cell growth, protein synthesis, and digestive and absorptive function which is crucial for calf survival (Guilloteau et al., 2009). In neonatal calves, the amount of ingested colostrum corresponds with small intestinal villus size (Blättler et al., 2001). Nutrient content, as well as non-nutrient components, of bovine colostrum affects villus size and mucosal cell proliferation (Roffler et al., 2003; Sauter et al., 2004). Therefore, the intestinal absorptive capacity increases in neonatal calves after colostrum intake as compared with calves fed either milk replacer or a milk-based formula with the same nutrient content as colostrum but almost no biologically active factors (Steinhoff-Wagner et al., 2011b).

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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 (Miyazaki et al., 2017). It is well known that the serum concentration of bioactive IgG is markedly increased after the first colostrum ingestion (Elsohaby and Keefe, 2015; Yang et al., 2015). However, little is known about the changes in blood small-molecule metabolites, such as carbohydrates, amino acids and lipids, in neonatal calves after the first colostrum ingestion, except for some blood biochemical parameters such as glucose, blood urea nitrogen (BUN), nonesterified fatty acids, and triglycerides (Hammon et al., 2002; Muri et al., 2005; Blum, 2006).

Metabolomics is a recent omics approach that involves studying the levels of small endogenous molecules or metabolites (Nicholson et al., 1999). These metabolites are either substrates or products involved in metabolic pathways (Claudino et al., 2007), and therefore, any changes in pathology or physiology might be reflected in the metabolotype (metabolic phenotype). Plasma metabolomics is used to monitor the health and metabolic status of calf herds (Ghaffari et al., 2017). ^1H nuclear magnetic resonance (NMR) spectroscopy is a method that can be applied to identify and quantify many metabolites in biological samples, including those in blood. The minimal sample preparation and high analytical reproducibility, along with the non-destructive characteristics of ^1H NMR spectroscopy, represent significant advantages of ^1H NMR in the field of metabolomics research compared with other methods (Emwas, 2015). Therefore, ^1H NMR-based metabolomics as a reliable method to determine a large number of blood metabolites simultaneously could further strengthen our understanding of physiologically healthy concentrations versus metabolic changes in neonatal calves.

We hypothesized that colostral small-molecule compounds are absorbed in the intestinal tract and transferred to the blood, resulting in a significant variation in plasma levels of such compounds and their metabolites before and after first ingestion of the colostrum in calves, as is the case for IgG. To test our hypothesis, a comprehensive analysis of plasma metabolites was performed before and after first colostrum ingestion in neonatal calves. In this study, ^1H NMR-based metabolomics analysis technology was used because it allowed us to study a large number of metabolites in the blood of neonatal calves. Moreover, metabolomics pathway analysis (MetPA) was used to visualize and interpret the

metabolic changes caused by the changes in metabolites concentrations. To the best of our knowledge, this is the first time that plasma metabolic profile has been characterized by ^1H NMR spectroscopy in neonatal calves fed the first colostrum. These findings will give new insights to understand better the metabolic response to the first colostrum feeding.

MATERIALS AND METHODS

Calves and Plasma Collection

This study was approved by the Animal Research Committee and followed the Animal Experiment Guidelines of Ningxia University. Eight healthy female Holstein calves, from a dairy farm in Yinchuan, China, with a mean body weight at birth of 43.4 ± 1.2 kg were used. All calves were singletons and spontaneously born to multiparous cows (2 to 3 parity) after normal-length gestation. The physical condition of each calf was evaluated based on appearance, vitality, and blood cell counts. These newborn calves were removed immediately from the pen to avoid suckling, and kept in single boxes with a room temperature of 20°C with straw bedding after calving.

Each calf was fed 4.0 L of colostrum coming from its corresponding dam using a rubber nipple attached to a bucket within 4 h after birth, because mixing colostrum from different cows was not allowed in the dairy farm. Colostrum from each cow was sampled and analyzed for quality by technicians of the Central Laboratory for Milk Recording (Yinchuan, China). Protein, fat and lactose contents in the colostrum were $15.20 \pm 0.20\%$, $5.10 \pm 0.15\%$, and $3.12 \pm 0.39\%$, respectively. Blood samples were taken by jugular vein into ice-cold polypropylene tubes containing heparin (10 IU/mL) as the anticoagulant before colostrum feeding (calves A, $n = 8$) and at 1 h after feeding (calves B, $n = 8$). Plasma samples were immediately prepared by centrifugation of the tubes at $1500 \times g$ for 10 min at 4°C , and the plasma collected was snap-frozen in liquid nitrogen and transferred to -80°C for storage until ^1H NMR analysis could be undertaken.

Sample Preparation and ^1H NMR Spectroscopy

The sample was prepared by mixing 180 μL of plasma with 360 μL of phosphate saline solution (0.045 M , $\text{K}_2\text{HPO}_4:\text{NaH}_2\text{PO}_4 = 4:1$, $\text{pD} = 7.4$, 0.9% NaCl) containing 50% D_2O , followed by transfer into a 5-mm micro-NMR tube.

One-dimensional ^1H NMR spectra were acquired for the plasma at 298 K on a Bruker Avance

III 600 MHz NMR spectrometer (Bruker BioSpin, Germany) equipped with a 5-mm TCI cryogenic inverse detection probe and use of a Carr–Purcell–Meiboom–Gill [CPMG, RD-90°-(τ -180°- τ) n acquisition] sequence with water presaturation (Zhao et al., 2012; Huang et al., 2013). A recycle delay time of 2 s, t_r of 11.5 μ s, and a fixed total spin–spin relaxation delay of 70 ms ($2n\tau$) were set. A total of 128 scans for plasma were collected into 32 K data points with a spectral width of 20 ppm.

¹H NMR Spectral Processes and Multivariate Analyses

The free induction decays were multiplied by an exponential window function with a ¹Hz line-broadening factor prior to Fourier transformation. ¹H NMR spectra were manually corrected for phase and baseline distortions by use of Topspin 2.1 (Bruker BioSpin). The anomeric proton of α -glucose with a chemical shift δ 5.233 was used as a spectral reference. All the ¹H NMR spectra were bucketed with spectral width of 0.004 ppm by use of AMIX software (version 3.9.2; Bruker BioSpin). The regions containing water (δ 4.5 to 5.15) were removed to avoid the effects of imperfect water suppression. The data of plasma spectra were imported into Simca-P 11.0 software (Umetrics, Malmö, Sweden) for multivariate analyses.

Principal component analysis (PCA) (Millsap, 1995) and orthogonal projection to latent structures discriminant analysis (O-PLS-DA) (Bylesjö et al., 2006) are 2 multivariate statistical analysis methods that are commonly used in metabolomics. PCA and OPLS-DA were used to reveal the global metabolic changes between calves A and calves B. The validity of the OPLS-DA model was certified by a 7-fold cross-validation method (Trygg and Wold, 2002), along with a permutation test method (Lindgren et al., 2015). The goodness-of-fit parameters for the OPLS model (R^2X , R^2Y , and Q^2Y) were calculated and varied from 0 to 1. R^2X and R^2Y represented the fraction of the variance of the x and y variables explained by the model, respectively. Q^2Y suggested the predictive performance of the model. The corresponding variable importance in the projection (variable importance projection [VIP] values) was also calculated in the OPLS-DA model. On the basis of a VIP threshold of 1, from the 7-fold cross-validated OPLS-DA model, some altered metabolite levels between calves A and calves B were observed. The altered metabolites with VIP >1 between the groups were all identified using a nonparametric Mann–Whitney *U* test (SPSS version 13.0, SPSS Inc., Chicago, IL), with the critical *P*-value set at 0.05.

Related Pathway Characterization

MetPA was used to expand the metabolomic analyses and to understand clearly the system-level effects of the metabolites (Xia and Wishart, 2016), which integrated pathway enrichment analysis and pathway topology analysis.

The significantly altered metabolites were imported into Metaboanalyst 3.0 (<http://www.metaboanalyst.ca/>) (Xia and Wishart, 2016). *Bos taurus* (cow) was selected as the model organism. An over-representation analysis (ORA) was applied for the functional enrichment analysis (Kaeffer et al., 2014). ORA was implemented using hypergeometric testing to evaluate whether a particular metabolite set was represented more than expected by chance within the metabolite sets. The one-tailed *P* values were calculated after adjusting for multiple testing (Benjamini and Hochberg procedure).

The pathway topological analysis was based on the relative between-ness and out-of-degree centrality measures of a metabolite in a given metabolic network, to calculate the importance of the metabolite (Abbasi et al., 2012). It measured the number of the shortest paths going through a node in the network and considered the global network structure and not just the immediate neighbor of the current node. The acquired impact value was the cumulative percentage of the importance of the matched metabolite nodes involved in a pathway.

Finally, the metabolome view was generated, which combined results from pathway enrichment analysis and pathway topology analysis. The y axis represented the negative log *P* value, original *P* value calculated from the enrichment analysis. The x axis represented the impact value from the pathway topology analysis. Potential targets were selected either by the *P*-values or impact (Wang et al., 2012). The impact value threshold was set as 0.10, and the negative log *P* value threshold was set at 10. Each different metabolite was cross-listed with the pathways in KEGG (Kyoto Encyclopedia of Genes and Genomes). The top level altered pathways were then identified and constructed, in accordance with the potential functional analysis.

RESULTS

Metabolite Assignments for ¹H NMR Spectroscopy

Representative proton ¹H NMR spectra of plasma obtained from calves A and calves B are illustrated in Fig. 1. Forty metabolite resonances were assigned according to the literature (Wang et al., 2005; Wang et al., 2007) and ¹H NMR

Figure 1. Representative proton ^1H nuclear magnetic resonance spectra of plasma from calves A and calves B. Key: 1, unsaturated fatty acids; 2, isoleucine; 3, leucine; 4, valine; 5, 3-hydroxyisobutyrate; 6, isopropanol; 7, 3-hydroxybutyrate; 8, lactate; 9, threonine; 10, lysine; 11, alanine; 12, acetate; 13, N-acetylated glycoproteins (NAGs); 14, glutamate; 15, methionine; 16, glutamine; 17, acetone; 18, acetoacetate; 19, succinate; 20, 2-ketoglutarate; 21, citrate; 22, creatine; 23, creatinine; 24, tyrosine; 25, malonate; 26, choline; 27, phosphorylcholine (PC); 28, glycerophosphocholine (GPC); 29, betaine; 30, taurine; 31, trimethylamine-*N*-oxide (TMAO); 32, β -glucose; 33, scyllitol; 34, methanol; 35, α -glucose; 36, glycine; 37, glycogen; 38, unknown; 39, histidine; 40, phenylalanine; 41, formate.

experiments. The plasma spectra revealed signals mainly from amino acids, unsaturated fatty acids (UFA), carboxylic acids, tricarboxylic acid (TCA) cycle intermediate metabolites (citrate, succinate, and 2-ketoglutarate), carbohydrates, and choline-containing metabolites. The detailed ^1H NMR assignments are presented in [Supplementary Table 1](#).

Metabolic Profiles of Calf A and Calf B

PCA was first performed to visualize the dataset that resulted from the ^1H NMR spectra of plasma from calves A and calves B and display the similarities and differences in scores plots ($R^2X = 0.542$, $Q^2 = 0.209$; [Fig. 2a](#)). Each sample was located inside the 95% confidence region of PCA model, and a separation trend was observed between Calves A (green circles) and calves B (blue squares). Furthermore, it was found that the OPLS-DA models indicated clear separation between calves A (green circles) and calves B (blue squares) ($R^2X = 0.565$, $R^2Y = 0.997$, $Q^2 = 0.89$;

[Fig. 2b](#)) because Q^2 was higher than 0.4. The results indicated significant alterations of plasma metabolic profiles between calves A and calves B.

Many of data points with a VIP threshold (>1) from the OPLS-DA model appeared to play an important role in the metabolic discrimination. Among 40 assigned metabolites, the VIP value of 36 metabolite resonances was >1 . The significance of the changes in concentrations was further validated with a nonparametric Mann–Whitney U test. The statistical parameters of the 36 metabolites in the 2 groups are listed in [Supplementary Table 2](#). Finally, 33 metabolites were identified as changed ($P < 0.05$; [Table 1](#)), including organic acids, amino acids, carbohydrates, and lipids. Among 33 metabolites, 19 were increased ($P < 0.05$), and the others were decreased after the first colostrum feeding ($P < 0.05$).

Characterization and Functional Analysis of the Key Metabolic Pathways

The relevant pathways for the 33 altered metabolites for Calves A and Calves B were visualized using

Figure 2. Principal component analysis (a) and orthogonal projection to latent structures discriminant analysis (b) scores plots for calves A (green circles) and calves B (blue squares).

Table 1. Metabolites that differed between calves A and calves B

Metabolite	VIP value ^a	Fold change ^{b,c}	Regulation ^d	<i>P</i> -value	Metabolites	VIP value ^a	Fold change ^{b,c}	Regulation ^d	<i>P</i> -value
2-Ketoglutarate	1.69	1.51	Down	1.76E-03	Isoleucine	1.45	-1.20	Up	4.53E-03
3-Hydroxybutyrate	1.54	-1.26	Up	2.44E-03	Isopropanol	1.37	10.63	Down	1.76E-03
3-Hydroxyisobutyrate	1.60	1.51	Down	2.44E-03	Lactate	1.10	1.76	Down	8.14E-03
Acetate	1.57	-1.16	Up	1.27E-03	Leucine	1.78	-1.31	Up	6.35E-04
Acetoacetate	1.62	-1.16	Up	6.36E-04	Lysine	1.30	-1.09	Up	1.08E-02
Acetone	1.15	-1.28	Up	4.53E-03	Methionine	1.66	1.40	Down	2.44E-03
Betaine	1.13	-1.44	Up	3.86E-02	NAG	1.14	1.16	Down	2.37E-02
Choline	1.61	-1.73	Up	1.76E-03	PC	1.06	-1.34	Up	3.86E-02
Citrate	1.29	1.14	Down	8.14E-03	Succinate	1.40	1.30	Down	1.08E-02
Creatine	1.30	-1.67	Up	1.08E-02	Taurine	1.38	1.63	Down	1.41E-02
Creatinine	1.73	1.84	Down	6.36E-04	Threonine	1.28	-1.09	Up	1.41E-02
Formate	1.02	-2.07	Up	8.14E-03	TMAO	1.50	-1.51	Up	2.37E-02
Glutamate	1.15	1.14	Down	2.37E-02	Tyrosine	1.67	-1.90	Up	9.01E-04
Glutamine	1.71	1.49	Down	8.94E-04	UFA	1.58	-1.63	Up	2.44E-03
Glycine	1.44	1.90	Down	1.76E-03	Valine	1.36	-1.45	Up	8.14E-03
GPC	1.02	-1.58	Up	3.86E-02	Glucose ^e	–	3.22	Down	9.00E-04
Histidine	1.57	-3.48	Up	1.27E-03					

^aVIP-value was calculated in the OPLS-DA model.

^bFold change was calculated as a ratio of the relative peak intensities between different groups.

^cPositive value, Calves A vs. Calves B; negative value, Calves B vs. Calves A.

^d“Up” or “Down” means the trend of regulation for these metabolites in Calves A vs. Calves B.

^eGlucose refers to the total of α -glucose and β -glucose, VIP of α -glucose is 1.09, VIP of β -glucose is 1.48.

GPC, glycerophosphocholine; NAG, N-acetylated glycoproteins; PC, phosphorylcholine; TMAO, trimethylamine-N-oxide; UFA, unsaturated fatty acids.

an interactive visualization framework (Fig. 3). [Supplementary Table 3](#) lists the results of the pathway analysis for Calves A and Calves B.

According to the impact values from the pathway analysis, 12 metabolic pathways for Calves A and Calves B were selected as the potential target pathways (impact value ≥ 0.10). Among these pathways, 2 biological modules were involved in lipid metabolism, including the synthesis and degradation of ketone bodies (impact = 0.60) and glycerophospholipid metabolism (impact = 0.10). Three biological modules were involved in carbohydrate metabolism, including glyoxylate and dicarboxylate metabolism (impact = 0.41), butanoate metabolism (impact = 0.10) and the TCA cycle (impact = 0.15). Seven biological modules were involved in metabolism of amino acids, such as glycine, serine, and threonine (impact = 0.29); valine, leucine, and isoleucine biosynthesis (impact = 0.10); phenylalanine, tyrosine, and tryptophan biosynthesis (impact = 0.50); histidine metabolism (impact = 0.27); alanine, aspartate, and glutamate metabolism (impact = 0.19); tyrosine metabolism (impact = 0.15); and taurine and hypotaurine metabolism (impact = 0.75). In addition, aminoacyl-tRNA biosynthesis was considered to be a potential target ($-\log P \geq 10$). These

results indicated that 13 metabolic pathways were significantly altered after the first colostrum in neonatal calves.

DISCUSSION

There is a report on 2-dimensional gas chromatography mass spectrometry (GC $\times$ GC-MS) based serum metabolomics in neonatal calves before and after first ingestion of colostrum ([Miyazaki et al., 2017](#)). That study found that 8 compounds, including oligosaccharides such as lactose, and some essential amino acids increased after ingestion. Nevertheless, the number of polar compounds detected by GC $\times$ GC-MS is limited. ^1H NMR-based metabolomics is a noninvasive and unbiased approach to identify metabolites ([Vicente-Munoz et al., 2015](#)). Considering the necessity to detect as many blood metabolites as possible for comprehensive nontarget metabolomics, we first applied ^1H NMR-based metabolomics to compare changes in plasma metabolic profiles and their corresponding metabolites in neonatal calves before and after first colostrum feeding. Forty metabolites were detectable with the CPMG [RD-90°-(τ -180°- τ)n acquisition] sequence in calf plasma samples by ^1H

Figure 3. Metabolomic map of significant metabolic pathways characterized in plasma for calves A and calves B. Light blue means the metabolite are not in our data but used as background for enrichment analysis; grey means the metabolite is not in our data and also excluded from enrichment analysis; other colors (varying from yellow to red), means the metabolites are in our data with different levels of significance for enrichment analysis; the original p value was calculated from the enrichment analysis; impact value is the pathway impact value calculated from pathway topology analysis. (a) valine, leucine and isoleucine biosynthesis; (b) taurine and hypotaurine metabolism; (c) synthesis and degradation of ketone bodies; (d) phenylalanine, tyrosine and tryptophan biosynthesis; (e) glyoxylate and dicarboxylate metabolism; (f) glycine, serine and threonine metabolism; (g) histidine metabolism; (h) alanine, aspartate and glutamate metabolism; (i) citrate cycle (TCA cycle); (j) tyrosine metabolism; (k) butanoate metabolism; (l) glycerophospholipid metabolism; (m) aminoacyl-tRNA biosynthesis.

NMR; of which 33 metabolites, including organic acids, amino acids, carbohydrates and lipids, were significantly altered, whereas only 11 compounds (including oligosaccharides and amino acids) were altered after first ingestion of colostrum with GC×GC-MS based metabolomics (Miyazaki et al., 2017). Therefore, ^1H NMR is a powerful for plasma metabolomics in calves.

A major finding of our study was that N-acetylated glycoproteins (NAGs) were significantly decreased after first colostrum feeding. N-acetylated glycoproteins are acute phase proteins (Deja et al., 2014) that increase at an early stage of inflammation, infection, neoplasia, stress, and trauma (Cray, 2012). Alteration of NAGs is not correlated with IgA, IgG, and IgM level (Torri et al., 1999). Calves immediately face stress resulting from new external environments after birth. Hence, the obvious decrease in plasma NAG concentrations after first colostrum feeding in this study, may have been in response to postnatal stress, and the first colostrum feeding could have had a positive effect on relieving the postnatal stress.

In this study, the first colostrum feeding improved the glucose status in neonatal calves by increasing glucose absorption that resulted in elevated plasma glucose. After birth, the glucose supply changes from prenatal parenteral via the placenta to lactose supply by colostrum and milk feeding (Hammon et al., 2013). Newborn calves have low plasma glucose concentrations (Steinhoff-Wagner et al., 2011a), but plasma glucose concentrations increase after the first meal (Hammon et al., 2003). In neonatal mammals, lactase hydrolyzes lactose into glucose and galactose, which are absorbed in the small intestine (Kehoe et al., 2007).

Lactate levels in the blood decreased in this study, which suggested that much lactate was used for gluconeogenesis to establish postnatal euglycemia after the first colostrum feeding. Lactose uptake does not meet glucose demand in the neonate, thus, maturation of the gluconeogenic pathway is necessary (Girard et al., 1992). Glycogenolysis and especially gluconeogenesis are important metabolic pathways to establish postnatal euglycemia (Girard, 1990). However, gluconeogenesis is absent or very low in the fetal liver when the mother is correctly fed, and the metabolic pathway emerges after birth to reach adult values after 24 h. Hence, gluconeogenesis increases rapidly in the liver of the newborn (Girard, 1990). Newborn ruminants can use lactate for gluconeogenesis (Donkin and Hammon, 2005).

In this study, not all amino acids increased after the first colostrum feeding, although amino acids may be derived from colostrum via amino acid transporters expressed in the epithelial cells of the small intestine (Miyazaki et al., 2017). Some amino acid including histidine, isoleucine, leucine, lysine, threonine, tyrosine, and valine increased, whereas alanine, as an important substrate for gluconeogenesis, as well as glycine, methionine, taurine, and glutamine decreased. This indicated that glycine, methionine, taurine, and glutamine, similar to alanine, were also used for gluconeogenesis so that their plasma concentrations decreased, because amino acids (especially alanine) are also used as substrates for gluconeogenesis within 8 h after birth (Girard et al., 1992).

In the present study, some intermediate metabolites of lipid metabolism, such as UFA, choline, phosphorylcholine, glycerophosphocholine, 3-hydroxybutyrate, and acetoacetate, increased after the first colostrum feeding. An increase in the level of these metabolites contributes to satisfying energy demand in neonatal calves. Due to fat intake by colostrum feeding, reflected by

increasing plasma concentrations of triacylglycerides and several proteins related to lipid transport in colostrum-fed calves (Herosimczyk et al., 2013), fat sources become an additional fuel to meet energy requirements in the neonate that requires adaptation of neonatal energy metabolism (Hammon et al., 2012). Another study found that blood concentrations of triglycerides, phospholipids, cholesterol, cholesterol-esters and linoleic acid sharply increase after milk feeding (Noble et al., 1975).

In this study, a decrease in intermediate metabolites of the TCA cycle including citric acid, succinic acid, and 2-ketoglutarate after the first colostrum feeding also indicated that neonatal calves need more energy to adapt to new environmental changes. Although calves receive glucose from lactose digestion with colostrum feeding, lactose uptake does not meet glucose demand in the neonate (Hammon et al., 2013).

Finally, a metabolomics pathway analysis was performed with MetPA to understand better the system-level metabolic response to the first colostrum feeding. A global metabolic response was reflected by alterations of 13 metabolic pathways including lipid, carbohydrate, and amino acid metabolisms in the neonatal calves.

Based on the foregoing analysis, we propose that besides meeting energy demand, a 4.0 L of high-quality colostrum feeding within 4 h after birth has a positive effect on relieving the postnatal stress in neonatal calves. Apparently, this study provides another perspective of response mechanisms of newborn calves upon the first colostrum feeding. Future research should focus on assessing the effects of prolonged colostrum feeding on metabolic profiles and relevant pathways in newborn calves, to confirm the consequence of colostrum feeding by metabolomics in combination with other biological methods.

SUPPLEMENTARY DATA

Supplementary data are available at *Animal Frontiers* online.

Conflict of interest statement. The authors declare that there are no competing financial and non-financial interests.

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