Gut Metabolomic Changes Before and After Parturition and Acquisition of a Microbiota

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Introduction

Mammalian fetuses under normal circumstances are believed to develop in a near-sterile uterine environment[1,2]. Microbial colonization of the ruminant gastrointestinal tract (GIT) begins during birth when it is exposed to a diversity of maternal and environmental microbes and progresses through until a climactic community establishes[3-6]. These microbes make significant contributions to the nutritional and immunological physiology of the animal[7,8]. Important microbial groups and critical nutritive functions such as hemicellulolytic (3 d), pectinolytic (3 d), cellulolytic (2-3 w), and methanogenic (1-3d) activities have been shown to emerge early in the rumen[3,4,9] but the complete compendium of biochemical consequences of these early microbiota on the gut remains unknown. To begin to quantify the biochemical influence of microbes we examined the GC-MS metabolomic profiles of GIT compartments and amniotic fluid from final trimester lamb fetuses (pre-microbial colonization) and compared those to metabolomic profiles of lamb rumen and feces collected following parturition (post-microbial exposure) from birth to 30 days of age. We hypothesized that postpartum samples will show a dramatic shift to a more complex metabolomic profile that exhibits microbial signatures.

Methods

- **Fetal Samples Were Collected From Dams In Their Final Trimester** Three final trimester fetuses were aseptically collected within 30 minutes of natural expiration of their dam. Amniotic fluid was immediately collected from intact placenta using sterile syringes and needles and transferred to sterile 15 ml falcon tubes and immediately frozen at -80 °C. The remaining fetuses were placed into sterile bags and frozen at -80 °C. Upon scheduling of a surgical unit at the MSU animal research center, collected fetuses were surgically dissected using strict aseptic techniques and samples of the rumen, abomasum, and meconium were obtained and stored sterile 15 ml falcon tubes and immediately frozen at -80 °C.
- **Lamb Rumen Tube And Rectal Swab Samples Were Obtained At Birth, 7, 14 And 30 Days Of Age** For comparison, rectal swabs and rumen samples were collected at birth and up to 30 days of age from lambs of the same breeding group. Rumen fluid was collected from lambs via autoclave-sterilized clear plastic stomach tubes designed for lambs/kids affixed to fresh sterile 50cc syringe. Individual lambs were assigned their own tubes to further avoid cross contamination. Samples were expelled into 15 ml falcon tubes, and frozen at -80 °C.
- **Samples Were Extracted And Centrifuged To Remove Particulate Matter** Samples were thawed slowly at 4 °C. Swab heads were cleaved from the swab stem using ethanol and flame sterilized scissors into fresh 15 ml falcon tubes containing 1.5 ml of ice-cold, sterilized molecular water and homogenized by vortex for 1 min. The homogenate was transferred to a fresh tube and 1.5 ml of 70% methanol was added to the tube containing the swab head and the extraction process was repeated with the resulting homogenate added to the water phase. All samples (swab and non-swab) were centrifuged at 21,000 x g at 4 °C for 10 min to remove particulate. Supernatants were transferred to sterile tubes, snap frozen and shipped to the Roy J. Carver Biotechnology Center (Urbana, IL) for Metabolomic profiling.
- **Small Molecule Metabolite Profiles Were Obtained By GC-MS** Samples were homogenized and equally divided in two. One fraction was derivatized by trimethylsilylation as previously described[10] the other was used to capture any naturally volatile compounds. The spectra of all chromatogram peaks were then compared with electron impact mass spectrum libraries NIST08 (NIST, MD, USA), WILEY08 (Palisade Corporation, NY, USA), and to a custom library of the Roy J. Carver Metabolomics Center. To allow direct comparisons between samples, all data was normalized to the internal standard in each chromatogram. The chromatograms and mass spectra were evaluated using the MSD ChemStation (Agilent, Palo Alto, CA, USA) and AMDIS (NIST, Gaithersburg, MD, USA).
- **Samples Were Analyzed To Determine Their Overall Relationship, The Portion Of Variation Explainable By By Age, Gut Location, And Animal, And To Identify Metabolites Unique to Postpartum Animals** Samples were visualized using a principal components analysis (PCA) and significant relationships determined by Permutational Multivariate Analysis of Variance (PERMANOVA) in Primer 6[11]. Metabolites not observed in placental samples were evaluated for the prevalence and abundance in lamb rumen and fecal samples for each age group.

- 276 Metabolites were detected across all samples. The numbers of metabolites seen in each sample did not differ by age ($P > 0.05$), but in postpartum lambs a greater number of metabolites were seen in rectal samples collected after day 0 than rumen samples ($P = 0.03$).
- The metabolomic profile of fetal samples did not vary between samples collected from the fetal abomasum, rumen, or rectum or from amniotic fluid (PERMANOVA P(perm) = 0.5)
- Fetal samples did differ from postpartum samples (PERMANOVA Pseudo-F = 35.7, P(perm) = 0.001) and this factor explained 42.5% of the total variation observed among samples.
- Postpartum lamb samples varied between rumen- and rectally- collected samples (PERMANOVA Pseudo-F = 7.2, P(perm) = 0.004) and this explained 19.1 % of the total variation in metabolome profiles among non-fetal samples.

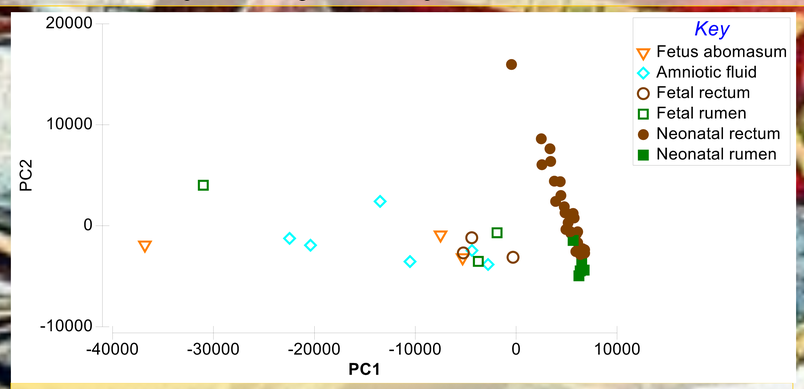

Figure 1. Fetal Samples Differ From Non-fetus Samples. PCA plot showing the relationships of all metabolome samples across the two major axes of variation.

Metabolite	Prevalence in PP Lamb Rumen	
	Days 7 - 18	Day 30
1. Shikimic acid*	100 %	100 %
2. Galactonic acid	100 %	100 %
3. 3-Methyl-4-hydroxybutyric acid	100 %	100 %
4. Hexanoic acid (C6:0)	100 %	75 %
5. Galactonic acid lactone	100 %	75 %
6. p-hydroxybenzaldehyde	50 %	100 %
7. Lauric acid (C12:0)*	50 %	100 %
8. Capric acid (C10:0)*	50 %	75 %
9. Deoxycholic acid*	50 %	75 %
10. Benzene-1,2,4-triol*	50 %	75 %
11. 1,3-Diaminopropane	50 %	75 %
12. Syringic acid	50 %	75 %

Table 1. Metabolites Not Seen In Fetal Samples But In > 75 % of Day 30 Rumen Samples. In total 30 metabolites not seen in any fetal samples were seen in one or more postpartum (PP) lamb rumen samples. Those listed above were seen in most rumen samples.

Metabolite	Prevalence in PP Lamb Rectum			
	Day 0	Day 7	Day 14	Day 30
1. Shikimic acid*	86 %	83 %	86 %	100 %
2. Phenylacetic acid	71 %	50 %	86 %	100 %
3. Hexanoic acid (C6:0)	100 %	83 %	100 %	88 %
4. Deoxycholic acid*	43 %	50 %	86 %	75 %
5. Cadaverine*	29 %	50 %	86 %	75 %
6. 3-Methyl-4-hydroxybutyric acid	57 %	50 %	57 %	75 %
7. Capric acid (C10:0)*	43 %	50 %	57 %	75 %
8. Galactonic acid	71 %	50 %	86 %	63 %
9. Syringic acid	57 %	100 %	71 %	63 %
10. Cholesta-3,5-diene	71 %	17 %	43 %	63 %
11. Butane, 1,2,4-trihydroxy	57 %	0 %	29 %	63 %
12. p-hydroxyhydrocinnamic acid	0 %	0 %	0 %	63 %

Table 2. Metabolites Not Seen In Fetal Samples But In > 60 % of Day 30 Rectal Samples. In total 67 metabolites not seen in any fetal sample were seen in one or more rectal sample. The metabolites listed above were seen in most rectal samples.

Conclusions

- Gut metabolome profiles were altered following birth leading to differentiation of fore- and hind-gut profiles
- Metabolites like Capric and Lauric acids likely reflect the introduction of colostrum and milk in the diet.
- Metabolites like Shikimic acid, Deoxycholic acid Cadaverine, and Benzene-1,2,4-triol likely reflect microbial metabolisms
- These data will provide a useful reference point for understanding the interactive metabolomic impact of diet and gut microbes and their influence on health and nutrition

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Literature Cited

1. Kemp et al. 2011. Reprod. Sci. 18: 88
2. Lauder et al. 2016. Microbiome 4: 29
3. Fonty et al. 1987. Microbiol. 133: 1835
4. Minato et al. 1992. J. Gen. Appl. Microbiol. 38: 447
5. Yáñez-Ruiz et al. 2010. FEMS Microbiol. Ecol. 72: 272
6. Jami et al. 2013. ISME J. 7: 1069
7. Round et al. 2009. Nat. Rev. Immunol. 9: 313
8. Yeoman & White. 2014. Annu. Rev. Anim. Biosci. 2: 469
9. Skillman et al. 2004. Anaerobe 10: 277
10. Yeoman et al. 2013. PLoS One 8: e56111
11. Clarke & Warwick. 2005. Nat. Environ. Res. Council.