Physiology and Endocrinology II

308 Methionine supply during late-gestation triggers offspring sex-specific divergent changes in metabolic and epigenetic signatures in bovine placenta. F. Batistel1, R. R. C. Yambao1, A. S. M. Alharthi1, Y.-X. Pan1, C. Parys2, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

Our objective was to investigate the impact of methionine supply during late-gestation on metabolism and DNA methylation in bovine placenta from cows carrying male or female calves. Multiparous cows were fed during the last 28 d of pregnancy a control diet or the control plus rumen-protected methionine (MET; Megron, Evonik Nutrition & Care GmbH; 0.09% of DMI) to achieve a 2.8:1 ratio of Lys:Met in the metabolizable protein. Placentome samples were collected from 15 cows/treatment and organized according to diet and offspring sex as follows: Male CTR (n = 7), Male MET (n = 7), Female CTR (n = 8), and Female MET (n = 8). Targeted metabolomics (LC-MS), RT-PCR, Western blotting, and enzyme activity were performed to quantify metabolic activity through the TCA cycle, 1-carbon metabolism, transsulfuration, and global DNA methylation. Compared with cows carrying Male CTR, cows carrying Male MET delivered heavier calves. Compared with placenta from Male CTR, Male MET placenta had greater concentrations of metabolites in the TCA cycle (isocitric acid and NADH) and transsulfuration pathway (cysteinesulfenic acid, glutathione and vitamin B12). Male MET placenta had greater methionine synthase (MTR) activity than Male CTR, while betaine-homocysteine S-methyltransferase (BHMT) and cystathionine-β-synthase (CBS) were not affected. No differences in global DNA methylation or mRNA and protein expression of the DNA methyltransferases were observed between Male CTR and Male MET. Cows carrying Female CTR and Female MET delivered calves with similar body weight at birth. Female MET compared with Female CTR placenta had greater concentrations of metabolites related to 1-carbon metabolism (Met and S-adenosyl-methionine). Enzyme activities in female placenta were not affected by methionine supply. The mRNA and protein expression of DNMT3A and DNMT3B was greater in Female MET than Female CTR, while DNMT1 was not affected by MET supply. Global DNA methylation was lower in Female MET than Female CTR placenta. Overall, our findings suggest that diet affects placental metabolism and DNA methylation and also highlight the importance of studying sex-specific responses to dietary interventions.

Key Words: amino acid, DNA methylation

309 Maternal supply of methionine during late-pregnancy affects hepatic one-carbon metabolism enzyme activity and plasma amino acids during the preweaning period in Holstein calves. A. S. M. Alharthi*1, P. Batistel1, C. I. M. Garces1, C. Parys2, Y.-X. Pan1, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

The objectives were to investigate if increasing supply of methionine during late-pregnancy in Holstein cows affects offspring plasma amino acid (AA) concentration and hepatic one-carbon (1C) metabolism. Calves were born to cows fed a control diet (CON) or the control plus rumen-protected methionine (MET) for the last 28 d prepartum (0.09% of DMI), and were fed and managed similarly after birth. Plasma samples and liver biopsies were harvested at 4, 14, 28, and 50 d of age and used for AA, mRNA expression and activity of hepatic 1C metabolism enzymes. Data were analyzed using a Mixed model considering block as random effect and treatment, time and its interaction as fixed effect. At birth, only the concentrations of plasma His and Met differed due to maternal treatment because of lower concentrations in MET calves. After birth, the overall concentrations of Met, Glu, and Leu was greater in MET compared with CON calves. There was an interaction of treatment×day (T×D) for the concentration of Tyr, Phe, Glu, Arg, Thr and Tau due to greater levels early in life in MET compared with CON calves. A T×D was observed for the activity of betaine-homocysteine S-methyltransferase (BHMT), methionine synthase enzyme (MTR), and cystathionine-β-synthase (CBS) in liver; MET calves had greater BHMT activity on d 14 and greater CBS on d 4 and peaked at 28. In contrast, despite a linear increase from d 4 to 28, activity of MTR in MET calves was lower on d 4 and 50. Methionine adenosyltransferase 1A (MAT1A) mRNA expression was greater overall in MET compared with CON calves. Greater mRNA expression of betaine-homocysteine S-methyltransferase 2 (BHMT2) and CBS in MET calves was observed on d 4 and 14. Along with CBS, the greater expression of cysteine dioxygenase 1 (CDO1) as well as glutathione reductase (GSR) and glutathione peroxidase (GPX1) on d 4, 14, and 28 in MET calves suggested greater synthesis of glutathione and taurine. Overall, the data indicate that enhanced methionine supply during the last 30 d of gestation not only can benefit calf performance, but increase AA availability and activity of the 1C metabolism pathway to furnish cells with antioxidants.

Key Words: glutathione, taurine

310 RNA sequencing reveals that methionine supply during late-gestation alters neonatal Holstein heifer calf liver transcriptome profiles. A. S. M. Alharthi*1, F. Batistel1, V. Palombo1, C. I. M. Garces1, C. Parys2, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

The objectives were to investigate if increasing supply of methionine during late-pregnancy in Holstein cows affects hepatic transcriptome profiles in female offspring. Heifer calves were born to cows fed a control diet (CON) or the control plus ethyl-cellulose rumen-protected methionine (MET) for the last 28 d prepartum (0.09% of dry matter intake), and were fed and managed similarly after birth. Liver biopsies were harvested at 4 d of age from 8 calves per treatment. Extracted total RNA was sequenced using the Illumina platform, and mapped to the Bos taurus genome assembly (UMD_3.1.1). Statistical analysis was conducted using the Bioconductor edgeR package, with treatment as fixed effect and animal as random effect. Dynamic Impact Approach (DIA) analysis was performed to uncover the most-impacted cellular pathways. A total of 622 differentially expressed genes (False Discovery Rate P < 0.25, uncorrected P < 0.01) were detected. The 25 most-impacted pathways from the DIA analysis indicated that genetic information processing pathways related with transcription and translation (e.g., RNA polymerase, purine metabolism, protein processing in endoplasmic reticulum, ribosome and spliceosome) were upregulated in MET than CON calves. In contrast, critical signaling pathways related to cell cycle, cellular metabolism and immunity (e.g., protein digestion and absorption, p53 signaling pathway, PI3K-Akt signaling pathway, Jak-STAT signaling pathway, calcium signaling pathway, cytokine-cytokine receptor interaction, cellular growth and death, cellular senescence, endocytosis and leukocyte transendothelial migration) were biologically more important and highly-upregulated in CON than MET.
calves. Overall, preliminary analysis indicated that molecular changes observed early after birth in response of maternal methionine supply during late-gestation are beneficial to overall hepatic function, especially in terms of metabolism and inflammatory status.

Key Words: fetal programming, liver, transcriptomics

311 Maternal supply of methionine during late-pregnancy alters the fecal microbiome in neonatal Holstein heifer calves during the preweaning period. A. Elolimy*,1, M. Zeineldin2, A. Allhalth3, F. Batistel4, A. Helmbrecht5, C. Parys6, and J. Loo1,4.

The objective of the current study was to investigate the impact of dietary methionine supply during late-pregnancy in dairy calves on gut microbiome and metabolome composition and their association with growth performance in neonatal calves from birth to weaning. Twenty-six Holstein heifer calves (n = 13/treatment) born to cows receiving a control diet (CON) or CON plus ethylcellulose rumen-protected methionine (MET, Mepron®, Evonik Industries AG, Germany) during the last 4 weeks of pregnancy. Calves received 3.8 L of first-milking colostrum from the respective dam within 8 h after birth. Calves were housed in individual outdoor hutches bedded with straw, fed twice daily with a milk replacer and had ad libitum access to a starter grain mix throughout the study. Fecal samples were collected at d 0 (i.e., at birth before colostrum feeding), 14, 28, and 42 (before weaning). Genomic DNA from fecal samples was used for amplification and sequencing of the V3-V4 hypervariable region of the 16S rRNA gene using Illumina MiSeq. Sequencing data were processed and analyzed with QIIME 2 software using the DADA2 pipeline. Calves from MET-fed cows had greater dry matter intake and average daily gain from birth to weaning. DNA from fecal samples was used for amplification and sequencing of the V3-V4 hypervariable region of the 16S rRNA gene using Illumina MiSeq. Sequencing data were processed and analyzed with QIIME 2 software using the DADA2 pipeline. Calves from MET-fed cows had greater dry matter intake and average daily gain from birth to weaning. DNA from fecal samples was used for amplification and sequencing of the V3-V4 hypervariable region of the 16S rRNA gene using Illumina MiSeq. Sequencing data were processed and analyzed with QIIME 2 software using the DADA2 pipeline. Calves from MET-fed cows had greater dry matter intake and average daily gain from birth to weaning.

Key Words: calves, microbiota, methionine

312 Maternal late-gestation metabolic stress is associated with changes in immune and metabolic responses of dairy calves. T. Ling6, M. Hernandez-Jover2,3, L. M. Sordillo1, and A. Abuelo*,1,3.

This study aimed to investigate if metabolic stress in late gestation dairy cows is associated with changes in the metabolic and immune responses of their offspring during the first month of life. Holstein-Friesian cows (n = 12) were blood sampled at 28 and 15 d before expected calving. The average between these 2 sampling points in the concentrations of nonesterified fatty acids (NEFA), haptoglobin (Hp), and oxidative stress index (OSi) were calculated as indicators of lipid mobilization, inflammation, and oxidative stress (OS), respectively. Calves received 4 L of colostrum from their respective dams within 12 h of life and were classified into groups (n = 6 each) according to their dams’ high or low degree of lipid mobilization, inflammation, and OS. The metabolic responses of calves in each of these groups were compared weekly up to 1 mo by assessing serum concentration of NEFA, Hp, and OSi. Additionally, whole blood at each sampling was subjected to a LPS-stimulated TNFα production assay to assess cell-mediated innate immunity against induced inflammatory responses. Mixed models with repeated measures were used. Calves born to cows with higher NEFA or OSi showed significantly lower birthweights, whereas no association between maternal metabolic stress groups and ADG was identified. Calves exposed to high maternal OS had higher concentrations of Hp (P = 0.013) and TNFα (P = 0.031), indicating greater basal inflammatory responses. In contrast, LPS-induced inflammatory responses were less robust in calves exposed to higher maternal Hp and OSi (Table 1), suggesting compromised immune responses. Collectively, these data suggest that prenatal exposure to maternal metabolic stress may adversely impact some metabolic and inflammatory responses of the offspring that could influence disease susceptibility.

Table 1 (Abstr. 312). TNFα release according to the degree of maternal OSi

<table>
<thead>
<tr>
<th>Week</th>
<th>10 ng/mL LPS stimulation</th>
<th>5 μg/mL LPS stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low OSi</td>
<td>High OSi</td>
</tr>
<tr>
<td>Wk 1</td>
<td>136.1±12.53</td>
<td>51.8±8.01*</td>
</tr>
<tr>
<td>Wk 2</td>
<td>117.2±11.47</td>
<td>24.3±4.55**</td>
</tr>
<tr>
<td>Wk 3</td>
<td>87.19±5.99</td>
<td>39.7±6.89*</td>
</tr>
<tr>
<td>Wk 4</td>
<td>72.9±2.92</td>
<td>32.0±5.62*</td>
</tr>
</tbody>
</table>

1Results expressed in percentage increase from control concentration (mean±SE).

Key Words: calf health, metabolic stress


The hypothesis of this study was that the exacerbated response of the innate immunity stimulated by pegbovigrastim when associated with a lipopolysaccharide challenge could negatively affect the liver, renal and protein metabolism of the dairy calves. Metabolic and hematological parameters were analyzed in dairy calves that received a dose of 0.25 μg/kg BW of *E. coli* lipopolysaccharide (LPS) associated with a dose of 25 μg/kg BW pegbovigrastim 24 h later. Twenty Holstein calves (D60 ± 15) were randomly distributed into 4 groups: LPS (n = 5) that received a single intravenous (IV) application of LPS (D0); PEG (n = 5), received a SC application of pegbovigrastim on d 1 (D1); PEG + LPS (n = 5), received a LPS injection D0 and pegbovigrastim at D1; and CTR group (n = 5), received a dose of 0.9% sodium chloride by IV at D0 and another SC dose at D1. For analysis of biochemical and hematological parameters, blood samples were collected on d –1, 0, 1, 2, 3, 4, 8, 14.
and 21. The biochemical parameters analyzed were albumin, aspartate aminotransferase, creatinine, gamma glutamyl transferase, PPT, urea, C-reactive protein, haptoglobin and activity of the paraoxonase 1 (PON1). Total leukocyte counts and other hematological variables were also analyzed at the same sampling points. Outcomes were analyzed using a repeated-measures ANOVA (Proc MIXED, SAS Studio). The LPS, PEG, and LPS + PEG groups showed an increase in the number of total leukocytes ($P < 0.0001$) in relation to the CTR, and the PEG and LPS + PEG groups remained with the highest number of cells from d 2 to d 21. The concentration of PON1 was lower in LPS + PEG in comparison to PEG ($P = 0.02$), but not different to the other groups. Moreover, the LPS + PEG group had higher GGT ($P = 0.0042$) e lower AST ($P < 0.0001$) and urea ($P = 0.02$) concentration that the other groups. Although some hepatic, renal and protein markers had been different in the LPS + PEG these were maintained in physiological concentrations. The results demonstrated that PEG injection even in LPS association increased the leukocytes for 21 d without compromising hepatic renal and protein metabolism.

**Key Words:** pegbovigrastim, immunity, paraoxonase (PON)

### 315 Embryonic development, luteal size and blood flow area, and metabolite of PGF$_{2\alpha}$ concentrations in dairy cows fed palm or sunflower oil supplement

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Benefits of dietary lipid supplementation during transition period have been shown to improve reproductive performance in dairy cows (Mattos et al., 2004; Thatcher et al., 2006). However, effect of different oil sources on reproduction has not been consistent (Funston, 2004). Thus, the objective was to examine effects of sunflower (SO) and palm oil (PO) supplements in diet on early embryonic development, luteal size and blood flow area, PGF$_{2\alpha}$ metabolite (PGFM), and progestrone (P4) concentrations. Prepartum cows ($n = 42$) were randomly assigned into one of 3 dietary treatments (control, 4% PO, and 4% SO supplements). Animals were fed diets individually from d 28 prepartum to d 111 postpartum. Cows were synchronized estrus with 2 injections of PGF$_{2\alpha}$ given 11 d apart. Blood samples were taken from d 5 until d 35 postpartum and throughout the estrous cycle (d 3, 5, 7, 9, 11, 13, 15, 17 and 19) for PGFM and P4 analyses, respectively. Luteal size and blood flow area were determined throughout the estrous cycle by Doppler ultrasonography. Oocytes were collected in 3 ovum pick-up sessions at 2-wk intervals for the in vitro embryo production starting on d 83 postpartum. Total DMI and milk yield were greater ($P < 0.05$) in cows fed plant oil supplements than control group during 35 d postpartum. Oocyte characteristics and embryonic development were not affected by dietary treatments. Cows fed 4% SO had greater ($P < 0.05$) concentration of PGFM from d 15 to d 35 postpartum than those cows fed 4% PO and control group. On the mid-luteal phase (d 11 of the estrous cycle), serum P4 concentrations (6.0 ± 0.7, 5.7 ± 0.5, and 4.7 ± 0.6 ng/mL), luteal size (7.0 ± 0.2, 6.5 ± 0.2, and 5.3 ± 0.1 cm$^2$), and luteal blood flow area (1.3 ± 0.2, 1.2 ± 0.1, and 0.9 ± 0.1 cm$^2$) were greater ($P < 0.05$) in cows fed 4% SO and 4% PO than control group, respectively. Thus, plant oil supplements in diet affected luteal size and serum P4 and PGFM concentrations, but not early embryonic development. Such changes in secretion of PGF$_{2\alpha}$ and P4 indicate that plant oil supplements during pre- and postpartum may alter uterine and luteal functions.

**Key Words:** corpus luteum, uterus, PGFM

### 316 Resynchronization treatments in dairy cows at non-pregnancy diagnosis based on corpus luteum status

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We tested (1) a shortened version of Ovsynch (OVS; GnRH1–7 d-PGF$_{2\alpha}$–24 h-PGF$_{2\alpha}$–32 h-GnRH2–16 h-AI) that excluded GnRH1 for resynchronization in cows bearing a corpus luteum (CL) at non-pregnancy diagnosis (NPD); (2) the value of including progesterone (P4) + OVS in absence of a CL compared with presence of a CL + OVS; and (3) the accuracy of detecting a functional CL by transrectal ultrasonography. Lactating Holsteins ($n = 1,589$) in 3 herds were enrolled in 3 treatments at NPD (32 ± 3 d after AI). Cows bearing a visually detected CL were assigned randomly to OVS or Short Synch (SS; PGF$_{2\alpha}$–24 h-PGF$_{2\alpha}$–32 h-GnRH2–16 h-AI), whereas cows with no CL were assigned to OVS + CIDR insert (CIDR). Blood collected at NPD (d 0) determined accuracy of treatment assignment based on P4 (functional CL cut point >1 ng/mL). In 1 herd, ovaries of 108 SS cows were scanned at d 0, 2 d after PGF$_{2\alpha}$ and 6 d after AI and on d 0, 7, 9, and 16 in OVS ($n = 97$) and CIDR ($n = 68$) cows to determine follicle diameter and ovulation risk. Treatment contrasts were made: OVS vs. CIDR and OVS vs SS. Ovulation risk after GnRH1 was greater ($P = 0.04$) for CIDR (40.3%) than OVS (27.1%) cows. Dominant follicle diameter before PGF$_{2\alpha}$ was greater ($P = 0.05$) for SS than OVS cows and P4 was less ($P < 0.01$) in CIDR compared with OVS cows. No differences were detected for luteolysis after PGF$_{2\alpha}$ (>96.6%) and ovulation risk after GnRH2 was 94.2, 91.7, and 85.2% for SS, OVS, and CIDR, respectively. Accuracy of treatment assignment was 59.6, 79.5, and 82.4% for CIDR, OVS, and SS cows, respectively. Technicians were more ($P < 0.01$) accurate in detecting a functional than non-functional CL. Pregnancy per AI (P/AI) in all cows was greater ($P = 0.03$) when P4 was ≥1 ng/mL at d 0. With herd as a random effect, P/AI was greater ($P = 0.02$) for CIDR (40.3%) than OVS (27.1%) cows. Dominant follicle diameter before PGF$_{2\alpha}$ was greater ($P = 0.05$) for SS than OVS cows and P4 was less ($P < 0.01$) in CIDR compared with OVS cows. No differences were detected for luteolysis after PGF$_{2\alpha}$ (>96.6%) and ovulation risk after GnRH2 was 94.2, 91.7, and 85.2% for SS, OVS, and CIDR, respectively. Accuracy of treatment assignment was 59.6, 79.5, and 82.4% for CIDR, OVS, and SS cows, respectively. Technicians were more ($P < 0.01$) accurate in detecting a functional than non-functional CL. Pregnancy per AI (P/AI) in all cows was greater ($P = 0.03$) when P4 was ≥1 ng/mL at d 0. With herd as a random effect, P/AI was greater ($P = 0.02$) for OVS than SS but did not differ from CIDR at d 29.6% [n = 644], 21.5% [n = 676], and 25.9% [n = 269], respectively. When cows within treatment were retrospectively categorized based on P4 cut point, P/AI did not differ among treatments (30.2% [OVS; n = 511], 27.4% [SS; n = 562], and 25.3% [CIDR; n = 164]). Short synch is a viable option when CL status can be accurately detected.

**Key Words:** artificial insemination, fertility, PGF$_{2\alpha}$

### 318 Hypothalamic metabolomics profiling in cattle with divergent residual feed intake

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The objective of the current study was to apply untargeted metabolomics profiling to determine potential hypothalamic metabolite signatures unique to the most and the least feed-efficient animals based on residual feed intake (RFI) classification. One hundred forty-nine Red Angus cattle were allocated to 3 groups according to herd origin. Animals were fed a finishing diet for 78 d to determine the RFI category for each. Within each contemporary group, the 2 most-efficient (n = 6; RFI coefficient = −2.69 ± 0.58 kg dry matter/d) and least-efficient animals (n = 6; RFI coefficient = 3.08 ± 0.55 kg dry matter/d) were selected. Hypothalamic
tissue was collected immediately after slaughter for metabolomics using a high-resolution mass spectrometry-based untargeted approach. Metabolites were analyzed using Q-Exactive MS system after LC separation. Data analysis was performed using the Metabo Analyst 4.0 program. Metabolites with 'importance in projection (VIP)' scores >1.0 and a 2-fold difference between groups were considered significantly different. The top 15 metabolites with highest VIP score were identified by molecular weight (mass error ppm <5) for comparison between groups. There were 47 distinct metabolite features identified and a clear discrimination between groups revealed through multivariate analysis (PLS-DA and OPLS-DA). Among the top 15 metabolites identified by the VIP analysis, succinic anhydride (VIP = 3.4), tris(butoxyethyl) phosphate (VIP = 2.9), and etioporphyrin III (VIP = 2.7) were greater in the most-efficient compared with the least-efficient animals. Because of the well-established physiologic role of hypothalamus on feed intake control, the data indicate that untargeted metabolomics profiling could be helpful for identifying RFI-specific biomarkers that may play a role in determining feed efficiency in cattle.

Key Words: residual feed intake (RFI), hypothalamus, metabolomics

319 The potential role of choline to alter histone methylation status revealed through a fluorescent protein system in bovine mammary epithelial cells. F. Rosa* and J. S. Osorio, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Dietary choline can be a source of methyl groups for histone methylation (HM), which can affect gene expression and consequently milk biosynthesis. Therefore, we evaluated the effect of choline on histone methylation in bovine mammary epithelial alveolar cells (MacT). Prior to transfection, cells were cultivated in high glucose Dulbecco modified Eagle’s medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone antimycotic. The plasmids used in this study were the pcDNA3-K9 and pcDNA3-K27 (Addgene) for analysis of HM through fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24h before transfection at 30,000 cells/well in a 96-well plate. Cells were transfected with Lipofectamine 3000 at 0.3 uL/well and at 50 ng/well of plasmid in a reduced serum medium (OptiMEM) deprived of FBS. Transfected cells were treated for 24h in triplicates with 0, 200, 400, and 800 ug/mL of choline. An inverted fluorescent microscope for live imaging (EVOS FL Auto) equipped with a motorized scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO2 was used to take 4 pictures/well at 4x magnification 0, 12, and 24h post-treatment. Transfection efficiency, viability, and quantification of HM were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS and significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.15$. Overall HM tend to decrease in K9 ($P = 0.13$) and increased in K27 ($P \leq 0.01$) during the 24h treatment. In K9 transfected cells, the 400 and 800 choline treatments maintain a HM status, whereas HM decreased ($P < 0.01$) over time in the control and 200 choline. In K27 transfected cells, the 200 choline treatment produced the greatest ($P < 0.01$) HM by 24h post-treatment. To expand on these effects, global DNA methylation and gene expression analysis will be performed. Our results indicate that choline can affect the HM status of histone tail residues (K9 and K27) differently, and this may be reflected in transcriptional changes and consequently in milk biosynthesis.

Key Words: choline, histone methylation, fluorescent proteins