Graduate Student Competition: ADSA/ASAS Northeast Graduate Paper Competition

101 Metabolomic profiling of the liver in developing chicken embryos and post-hatch chicks reveals unique metabolic differences. Q. Hu,* U. Agarwal, and B. J. Bequette, *University of Maryland, College Park.*

The emerging new field of metabolomics aides in the identification and characterization of global metabolite patterns of organs and tissues. In this study, a metabolomic profiling approach was employed to investigate differences in metabolism in the liver of chicken embryos from 2 egg sizes and from broiler breeders of different maternal ages. Whole livers were collected on embryonic (e) d 17 and 20, and on post-hatch d 1 (n = 9–10) from embryos and chicks derived from broiler breeders of different ages (32 wk vs 51 wk, 63.2 ± 1.2 g) and from 2 sizes of eggs laid by 45 wk old breeders (55.8 ± 1.2 g vs 67.7 ± 1.1 g). Livers were lyophilized to dryness and freezer-milled, and the metabolites were extracted before chemical derivatization. Metabolites were separated by gas chromatography and under full scan mode complete ion spectra were recorded by mass spectrometry. Data files were converted using Agilent data analysis software and processed with the XCMS online server. Compound identification was determined by searching against the NIST 2008 library. Principal component analysis was employed to visualize the metabolite differences between 2 groups at 3 developmental stages. The results showed that embryos on both e17 and e20 from 32 wk old breeders clustered separately from 51 wk old breeders. However, this was not observed for embryos from small vs large eggs at these developmental stages. Concentrations of 6 metabolites from e17 livers differed ($P < 0.05$) between 32 wk and 51 wk old breeder eggs, 13 metabolites differed on e20 and 5 metabolites differed on post-hatch d 1. Metabolite categories included amino acids, carbohydrates, fatty acids and cholesterol esters. Comparison of small and large egg sizes revealed that 14 metabolites differed ($P < 0.05$) on e17, 13 metabolites differed on e20 and 6 metabolites differed on post-hatch d 1. In conclusion, these results reflect that the liver metabolisms of embryos during later development are distinct due to breeder age and egg size. Together, this study is the first assessment of global metabolism of developing embryo livers and our data provides the framework for further metabolic pathway analysis.

Key Words: chicken embryo, liver, metabolomic profiling

102 Effect of resveratrol supplementation on glycemic response in moderately exercised geldings. J. L. Zambito*[1], H. S. Spooner[1], and R. Hoffman[2], *[1]West Virginia University, Morgantown, [2]Middle Tennessee State University, Murfreesboro.*

Resveratrol is known to exert numerous health benefits including improved glycemic response. Resveratrol is present in equine supplements, yet no research exists in an athletic horse model. This study tested the hypothesis that resveratrol supplementation would improve glucose tolerance and insulin sensitivity in the athletic horse. Six mature geldings were assigned to 3 groups in a Latin Square design. Horses either received no supplementation (control, C) or one of 2 doses of trans-resveratrol (Equithrive, Lawless, KY); low (L, 2.5g) or high (H, 5g) daily for 14-d. Horses were exercised 3x weekly for 60 min. Body weight (BW) was collected on d0 and d14 of each period. A frequently sampled intravenous glucose tolerance test (FSIGT) was conducted on d10. Samples were analyzed for plasma insulin (INS) and glucose (GLU). Area under the curve (AUC) for INS and GLU was calculated via the trapezoidal method. Glucose effectiveness (Sg), insulin sensitivity (Si), acute insulin response to glucose (AIRg) and disposition index (DI) were calculated using minimal model software. Data were analyzed using ProcGLM in SAS 9.1, and further separated by Tukey's test. Contrasts were conducted to examine differences between C and treatment (T, both low and high). Minimal model analysis of FSIGT, along with AUC INS and GLU showed no effect of resveratrol supplementation (Table 1). In conclusion, resveratrol supplementation in the moderately exercised horse did not improve glycemic response, which may already be optimized in this model. These findings indicate a need to further evaluate resveratrol in other equine models, including a more in-depth assessment in the athletic horse.

Table 1. Glucose and insulin parameters in exercising horses supplemented with resveratrol

	Treatment			*P*-value	
	Control	Low	High	Treatment	Control
Sg (min^{-1})	0.030 ± 0.003	0.032 ± 0.004	0.035 ± 0.003	0.56	0.43
Si ($L*mU^{-1}*min^{-1}*10^{-4}$)	5.93 ± 1.77	7.83 ± 2.33	8.70 ± 1.77	0.59	0.38
AIRg (mU*min*L^{-1})	304 ± 21	333 ± 28	272 ± 21	0.34	0.96
DI	2092 ± 647	2204 ± 857	2440 ± 648	0.93	0.80
AUC GLU (mg/dL*min)	20433 ± 1241	19302 ± 1241	19506 ± 1241	0.80	0.54
AUC INS (mIU/L*min)	7057 ± 992	6666 ± 992	6213 ± 992	0.84	0.64

Key Words: horse, exercise, resveratrol

103 Effects of intrauterine growth retardation due to poor maternal nutrition on bone formation in sheep. S. Neupane*[1], M. L. Hoffman[1], M. A. Rokosa[1], E. R. Ackell[1], D. M. Kaelin[1], S. A. Zinn[1], T. D. Crenshaw[2], and K. E. Govoni[1], *[1]Department of Animal Science, University of Connecticut, Storrs, [2]Department of Animal Science, University of Wisconsin, Madison.*

Intrauterine growth retardation (IUGR), often due to poor maternal nutrition, leads to either slow growth or reduced fetal size. Specifically, IUGR impairs muscle and bone development, and leads to increased adipose tissue development. We hypothesized that poor maternal nutrition would impair bone formation in offspring by reducing the differentiation of bone marrow stromal cells (BMSC) into osteoblasts. A total of 24 ewes were fed a diet of 60% (R; n = 8), 100% (C; n = 8), or 120% (O; n = 8) of NRC requirements beginning at d 85 of gestation. Lambs were euthanized at birth (n = 3/treatment) and 3 mo of age (n = 3 to 5/treatment). Femurs (F) and tibia (T) were collected at birth and 3 mo and BMSC were collected at birth. Bone mineral content (BMC) and bone mineral density (BMD) were measured on excised bones by dual-energy x-ray absorptiometry. To determine if IUGR affects ability of BMSC to differentiate into osteoblasts, BMSC were cultured in differentiation media (α-MEM plus β-glycerophosphate, dexamethasone and ascorbic acid) for 21 d and stained with alizarin red to determine mineralization. As reported, BW were reduced in R lambs by 16% at birth and 3 mo ($P \leq 0.05$) compared with C. At birth, maternal diets tended ($P = 0.19$) to affect bone area (22.6 ± 2.7, 20.6 ± 3.4, and 26.0 ± 1.3 cm^2; C, R and O,

respectively); however, significant treatment effects were not observed for F BMC or BMD ($P \geq 0.29$). An effect of treatment was not observed for T variables at birth ($P \geq 0.29$). At 3 mo, bone area of the T tended ($P = 0.10$) to be reduced in R and O groups compared with C (53.8 ± 0.7, 50.8 ± 1.5, 50.7 ± 0.6 cm^2; C, R, and O, respectively). Compared with C, significant differences in BMC and BMD were not detected for F or T at 3 mo ($P \geq 0.51$). Cell culture experiments did not demonstrate a significant difference in alizarin red staining between treatment groups ($P \geq 0.6$). In conclusion, poor maternal nutrition reduces bone area in offspring with effects persisting during early postnatal development.

Key Words: bone, bone marrow stromal cells, intrauterine growth retardation

104 Hypoxia stimulates GLUT1 expression in bovine mammary epithelial cells. Y. Shao*[1], K. M. Lounsbury[2], T. L. Wellman[2], and F.-Q. Zhao[1], *[1]Laboratory of Lactation Physiology, Department of Animal Science, University of Vermont, Burlington, [2]Department of Pharmacology, University of Vermont, Burlington.*

Expression of glucose transporters in bovine mammary gland increases a few to several hundred-fold from late pregnancy to early lactation and this increase is not mediated by lactogenic hormones. Hypoxia has been shown to increase glucose transporter 1 (GLUT1) expression in many cell types. Deletion of hypoxia-inducible factor HIF-1α, a key signaling factor which mediates hypoxia effects, in mouse mammary gland results in impaired mammary differentiation and failure in lactation. Therefore, we hypothesized that hypoxia mediates the upregulation of GLUT expression in bovine mammary gland during the transition period. To test our hypothesis, Mac-T bovine mammary epithelial cells and bovine primary mammary epithelial cells were treated with 2% of O_2. GLUT1 mRNA increased 2.2-, 2.8-, and 2.9-fold in Mac-T cells and 5.1-, 8.3-, and 4.2-fold in primary cells after 3, 12, and 24 h hypoxia treatment, respectively ($P < 0.01$). However, GLUT8 mRNA decreased by 42% and 30% in Mac-T cells and 50% and 51% in primary cells after 12 and 24 h hypoxia treatment ($P < 0.01$). Then Mac-T cells were treated with 2, 5, and 10% of O_2 for 12h. GLUT1 mRNA increased 2.4- and 1.8-fold ($P < 0.05$) in cells treated with 2% and 5% of O_2 respectively, and 10% O_2 had no effect. In addition, Mac-T cells and primary cells were treated with 2% of O_2 for 24 h and then incubated with 200 μM of 2-NBDG, a glucose analog with fluorescent group, for 15 min. Glucose uptake was measured by flow cytometry and results showed that hypoxia increased glucose uptake 2.1 fold in Mac-T cells and 2.8 fold in primary cells ($P < 0.05$). Furthermore, when Mac-T cells were transfected with HIF-1α siRNA for 6 h and then treated with 2% of O_2 for 12 h, the upregulation of GLUT1 by hypoxia was abolished, indicating that hypoxia regulates GLUT1 expression through HIF-1α in bovine mammary epithelial cells. Our studies suggest that hypoxia, associated with high levels of oxygen consumption in mammary development and lactogenesis, may be at least partially responsible for increasing GLUT1 expression and glucose uptake through transcription factor HIF-1α in bovine mammary epithelial cells during the transition period.

Key Words: hypoxia, glucose transporter, mammary gland

105 Poor maternal nutrition reduced body weights and circulating concentrations of IGF-I and IGFBP-3 in lambs. M. A. Rokosa,* M. L. Hoffman, S. Neupane, K. E. Govoni, A. M. Bush, T. A. Hoagland, and S. A. Zinn, *Department of Animal Science, University of Connecticut, Storrs.*

Intrauterine growth retardation (IUGR) may be the result of poor maternal nutrition, and can influence the somatotropic axis and the growth potential of offspring. To determine the effects of nutritional status during gestation on growth and serum concentrations of IGF-I and IGFBP-3 in lambs, 24 (21 Dorsets, 3 Shropshires) ewes were assigned (balanced for breed, parity and age) to 1 of 3 diets; control (C; 100% NRC, n = 8), restricted (R; 60% NRC, n = 8), or overfed (O; 120% NRC, n = 8). Treatment started at d 85 ± 5 of gestation. Nine lambs (3/treatment) were slaughter within 24 h of birth (d 1) and 15 lambs (3 to 5/treatment) were fed a control diet consisting of milk replacer (fed at 1.7%/BW), creep feed and hay for 3 mo and then euthanized. Body weights were taken on d 1 and every 2 to 4 d until slaughter. Crown rump (CRL) measurements were taken weekly, and heart girth (HG) measurements were taken at d 1 and slaughter. Blood samples (10 mL) were collected on d 1 and every 7 d until slaughter. Concentrations of IGF-I and IGFBP were determined by RIA and ligand blot, respectively. At birth, lambs from R ewes (4.15 ± 0.3 kg) were lighter than C (5.3 ± 0.3 kg) and O (5.1 ± 0.3 kg) lambs ($P = 0.01$). At 3 mo, lambs from R ewes (31.0 ± 1.3 kg) tended to be lighter ($P = 0.06$) than C (35.2 ± 1.3 kg) and O (33.5 ± 1.7 kg) lambs. However, we did not observe an effect of maternal diet on CRL at birth ($P = 0.67$) or 3 mo of age ($P = 0.29$). At birth, heart weight ($P = 0.06$) and HG ($P < 0.01$) were less in R (30.2 ± 3.2 g and 36.9 ± 0.8 cm, respectively) than C (36.3 ± 3.2 g and 40.7 ± 0.8 cm, respectively) and O (43.8 ± 3.2 g and 40.4 ± 0.8 cm, respectively) lambs, but not at 3 mo of age ($P = 0.60$, $P = 0.17$). Average serum IGF-I was decreased ($P = 0.04$) in R (158 ± 36 ng/mL) compared with C (308 ± 37 ng/mL) and O (227 ± 40 ng/mL) lambs. Average concentrations of IGFBP-3 were decreased ($P = 0.05$) in R (1,032 ± 135 AU) and O (1,014 ± 148 AU) compared with C (1,438 ± 135 AU). We did not observe a treatment effect on circulating IGFBP-2 ($P = 0.63$). In conclusion, poor maternal nutrition may inhibit fetal growth by reducing circulating concentrations of IGF-I and IGFBP-3.

Key Words: intrauterine growth retardation, IGF, IGFBP

106 Effect of rumen-protected amino acid supplementation of a protein-deficient diet on performance of lactating dairy cows. C. Lee*[1], A. N. Hristov[1], T. Cassidy[1], K. Heyler[1], H. Lapierre[2], G. A. Varga[1], M. J. de Veth[3], A. Patton[4], and C. Parys[5], *[1]The Pennsylvania State University, University Park, [2]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [3]Balchem Corporation, New Hampton, NY, [4]Nittany Dairy Nutrition Inc., Mifflinburg, PA, [5]Evonik Industries AG, Hanau, Germany.*

The objective of this experiment was to evaluate the effect of supplementing a metabolizable protein (MP)-deficient diet with rumen-protected (RP) Lys, Met, and specifically His on dairy cow performance. The experiment was conducted for 12 wk with 48 Holstein cows (75 ± 5.6 DIM). Following a 2-wk covariate period, cows were blocked by DIM and milk yield and randomly assigned to one of 4 diets, based on corn silage and alfalfa haylage: control, MP-adequate diet (AP; MP balance: +9 g/d); MP-deficient diet (DP; MP balance: −317 g/d); DP supplemented with RPLys (AminoShure-L) and RPMet (Mepron; DP-LM); and DP-LM supplemented with RPHis (DP-LMH). Analyzed CP content of the AP and DP diets was 15.7 and 13.6%. Apparent total tract digestibility of all nutrients, plasma urea-N, and urinary-N excretion were decreased ($P < 0.001$) by the DP diets compared with AP. Milk N secretion as a proportion of N intake was greater (34.6 vs. 29.4%, $P = 0.001$) for the DP diets compared with AP. Compared with AP, DMI tended to be lower ($P = 0.06$) for DP, but was similar for DP-LM and DP-LMH (24.5, 23.0, 23.7, and 24.3 kg/d, respectively). Milk yield was decreased ($P = 0.004$) by DP (35.2 kg/d), but was similar to AP (38.8 kg/d) for the DP-LM and DP-LMH diets (36.9 and 38.5 kg/d, respectively), following the trend in DMI. Milk fat and true protein

content did not differ among treatments. Milk protein yield followed the trend in milk yield response ($P = 0.002$; 1.13, 1.01, 1.10, and 1.14 kg/d, respectively). Plasma essential AA, Lys, and His were lower ($P < 0.03$) for DP compared with AP. Compared with AP, plasma Met concentration was higher ($P < 0.001$) for DP-LM and DP-LMH and that of His was similar for DP-LMH. In conclusion, MP-deficiency, approximately 15% below NRC (2001) requirements, decreased DMI and milk yield in dairy cows. Supplementation of the DP diet with RPLys and RPMet diminished the difference in milk yield compared with AP and addition of RPHis eliminated it. This study suggests that His is likely a limiting AA in dairy cows fed MP-deficient diets.

Key Words: metabolizable protein, rumen-protected histidine, dairy cow

107 Effects of lasalocid and pulse-dosed chlortetracycline on health, growth, and thyroxine concentrations of prepubertal dairy heifers. R. Cabral*[1], P. Erickson[1], N. Guindon[1], E. Kent[1], C. Chapman[1], K. Aragona[1], M. Cabral[1], E. Massa[1], and M. Branine[2], *[1]University of New Hampshire, Durham, [2]Pfizer Animal Health, Canon City, CO.*

The effects of feeding chlortetracycline (CTC) and lasalocid (L) and the combination were studied using 40, 12 wk old Holstein heifers (108 ± 12 kg) in a randomized complete block design utilizing a 2 × 2 factorial arrangement of treatments. Heifers were blocked by birth date to 1 of 4 treatments: 1) carrier (30 g; C); 2) L + carrier (1 mg/kg BW; L); 3) CTC+ carrier (22 mg/kg BW; CTC); 4) L + CTC + carrier (CTCL). Heifers on CTC/CTCL were given their respective treatment during wk 1–4, 6, and 10 and within these weeks given treatment Monday— Friday and carrier only on weekends. Heifers were individually fed a total mixed ration (TMR) with treatments top-dressed at 1200 h daily. Heifer DMI was monitored and adjusted according to individual intakes. Skeletal measurements were taken weekly and blood samples obtained every Monday, Wednesday, and Friday. Blood samples were analyzed for thyroxine concentration via radial immunoassay. Heifers supplemented with L (L, CTCL) had lower DMI ($P = 0.05$), ADG ($P = 0.02$), overall BW gain ($P = 0.03$), overall wither gain ($P = 0.04$), and overall girth gain ($P = 0.09$) compared with heifers not fed L (C, CTC). Overall BW ($P = 0.04$) and body length ($P = 0.02$) gains and ADG ($P = 0.10$) were greater in CTC heifers (CTC, CTCL). Interactions occurred for: feed efficiency ($P = 0.03$), overall hip height ($P = 0.03$), and wither height ($P < 0.01$) gains and daily hip height gains ($P = 0.08$) were greater in C and CTCL heifers compared with CTC and L heifers. There was no effect on serum thyroxine concentrations. Supplementation with L did not increase growth. Supplementation with CTC improved growth for some skeletal measurements. The combination of CTC and L was more effective than either L or CTC fed individually. Supplementing CTC and L to growing heifers under conditions of stress may be of benefit.

Key Words: heifer, lasalocid, chlortetracycline

108 Effect of post-ruminal supplementation of phytonutrients on immune response, blood cell counts, and blood chemistry in lactating dairy cows. J. Oh*[1], A. N. Hristov[1], C. Lee[1], K. Heyler[1], T. Cassidy[1], J. Pate[1], S. Walusimbi[1], E. Brzezicka[1], K. Toyokawa[1], J. Werner[1], and D. Bravo[2], *[1]The Pennsylvania State University, University Park, [2]Pancosma, Geneva, Switzerland.*

The objective of this experiment was to investigate the effects of post-ruminal supplementation of phytonutrients on immune response, blood cell counts and blood chemistry in lactating dairy cows. Eight ruminally-cannulated Holstein cows (232 ± 34.1 d in milk) were used in a replicated 4 × 4 Latin square design trial with 23-d periods. Treatments were: (1) control (CON), (2) 2 g/d curcuma oleoresin (CU), (3) 2 g/d garlic extract (GE), and (4) 2 g/d capsicum oleoresin (CA). The phytonutrients were dissolved in ethanol solution and pulse-dosed into the abomasum of the cows once daily, 2 h after feeding for 9 d during each experimental period. Control cows received ethanol solution only. Compared with CON, CU, GE, and CA increased ($P = 0.01$) concentration of cluster of differentiation-antigen-4-positive cells ($CD4^+$; 11.9 vs. 20.3, 18.4, and 17.8%, respectively). Treatments had no effect on $\gamma\delta^+$ and $CD8^+$ cells, interleukin-6, tumor necrosis factor-α and interferon-γ cytokines, and T-cell proliferation ($P = 0.34$ to 1.00). Monocyte counts were lowered ($P = 0.04$) by CU and GE compared with CON (0.26, 0.26 and 0.33 × 10^3/μL, respectively). Relative to CON, CA increased ($P = 0.04$) lymphocytes as proportion of the total white blood cells (WBC; 39.3 and 41.8%, respectively). Lymphocyte counts tended to be decreased ($P = 0.10$) by GE compared with CON (3.43 and 3.77 × 10^3/μL, respectively). Cows treated with GE and CA had lower ($P = 0.03$) mean platelet volume than CON cows (6.51, 6.56, and 6.91 fL, respectively). Blood glucose, BUN, and creatinine concentrations were not different among treatments ($P = 0.39$ to 0.47). In conclusion, post-ruminal supplementation of phytonutrients had no effect on $\gamma\delta^+$ cells, $CD8^+$ cells, cytokine concentrations, T-cell proliferation, and blood chemistry, but all treatments significantly enhanced $CD4^+$ cells, CU and GE reduced monocytes, and CA increased lymphocyte proportion of WBC in dairy cows.

Key Words: phytonutrients, immune response, dairy cow