829 Assessing heat load and dissipation using digital infrared thermography and serum cortisol profiles in horses during the summer months. Y. Dupre[1], A. Strohm[2], E. Keis[2], J. Harney[2], K. Moulton*[2], and P. L. Ryan[2], [1]*Tuskegee University*,[2]*Mississippi State University*.

During the summer months in southeastern United States environmental factors including high ambient temperature, high relative humidity and radiant energy contribute to heat stress in cattle, but little is known about this condition in horses. The objectives of this study were to evaluate heat load and dissipation in horses during the summer, determine whether coat color is a factor, and assess physiological responses by monitoring systemic cortisol. Fifteen mares (3–18 years) were assigned to one of 5 coat color categories (n = 3/group; bays, browns, gray, paint, palomino) and maintained on pastures with the same shade cover. Heat load was assessed using digital infrared thermal imaging (FLIR T400 camera) and rectal temperatures were recorded 4x/d (0600, 1200, 1600, 2000 h), 2 d/week for 6 weeks (June–July, 2009) along with ambient temperature, wind speed and humidity. Five regions of interest (ROI; flank, shoulder, eye, muzzle, perineum) were imaged at each time point. Minimum (MIN), maximum (MAX), average (AVE) and standard deviation (STDEV) thermal signatures were assessed for each ROI. Blood was collected at 0600, 1600 and 2000 h for serum cortisol. Data was analyzed using GLM procedures of SAS. LS means were calculated and separated using Fisher's LSD ($P < 0.05$). Bay and brown horses had the highest thermal signatures (AVE/MAX) at 1200 and 1600 h in all ROIs except the perineum and eye compared with all other groups ($P < 0.05$). Shoulder and flank regions had the highest AVE/MAX (~40°C) compared with other ROIs. For combined ROIs, bay/brown mares had the highest MAX value ($P < 0.01$). Grey mares had the greatest perineum MAX value (37.15–37.7°C) throughout the day ($P < 0.05$) compared with other groups (34.32–37.56°C) as well as the highest rectal temperature ($P < 0.05$). Serum cortisol was lower in paint horses ($P < 0.05$) compared with other coat colors at each time point, and declined from a high at 0600 to a low at 2000 h ($P < 0.05$) in all coat groups. In conclusion, coat color affects heat load and dissipation in horses, which is influenced by the darker color consistent with heat absorption. Lower serum cortisol was consistent with low thermal signatures in paint horses (pale skin pigment).

Key Words: heat stress, thermography, equine

830 Effects of selenium supplementation and prolonged exercise on antioxidant gene expression in equine skeletal muscle. S. White*, L. K. Warren, S. E. Johnson, and J. Bobel, *University of Florida*, *Gainesville*.

Twelve untrained Thoroughbred horses (mean ± SE; 11 ± 1 y; 565 ± 11 kg) were used to evaluate antioxidant gene expression in skeletal muscle in response to prolonged exercise after receiving 2 levels of dietary selenium (Se). Horses were randomly assigned to diets containing either 0.1 mg Se/kg DM (n = 6; CON) or 0.3 mg Se/kg DM (n = 6; SE) for 36 d. Horses were individually fed 1.6% BW/d of coastal bermudagrass hay (0.02 mg Se/kg DM), 0.4% BW/d of whole oats (0.24 mg Se/kg DM) and a mineral/vitamin premix containing no Se. Sodium selenite was added to achieve either 0.1 or 0.3 mg Se/kg DM in the total diet. On d 35, horses underwent 2 h submaximal exercise in a free-stall exerciser (total distance 26 km; heart rate 135 ± 39 bpm). Biopsies of the middle gluteal muscle were obtained before Se supplementation was initiated, before exercise, and at 6 and 24 h post-exercise. Gene expression in skeletal muscle was determined by quantitative RT-PCR using 18S as a reference gene. Differences in mRNA expression due to treatment and exercise were analyzed using the GLIMMIX procedure of SAS (v. 9.2). Muscle expression of *MT1B* ($P = 0.001$) and *MT3* ($P = 0.001$) both increased over 6-fold from pre- to 24 h post-exercise, indicating exercise was sufficient to elicit oxidative stress. Muscle expression of *TrxR1* was unchanged at 6 h but increased ($P = 0.02$) 2.5-fold at 24 h post-exercise, whereas *GPx1* and *GPx3* did not change through 24 h post-exercise. Expression of *SOD1* and *SOD2* were unchanged through 6 h post-exercise, but expression of both genes increased ($P = 0.001$ *SOD1*; $P = 0.0003$ *SOD2*) in CON horses and *SOD2* decreased ($P = 0.04$) in SE horses from 6 to 24 h post-exercise. Level of dietary Se had no overall effect on expression of *MT1B*, *MT3*, *TrxR1*, *GPx1*, *GPx3*, *SOD1*, or *SOD2* in muscle following exercise. Prolonged exercise in untrained horses appears to upregulate mRNA expression of some antioxidant enzymes in skeletal muscle. Short-term Se supplementation above the current NRC requirement did not alter selenoprotein expression in skeletal muscle, but may influence expression of other antioxidant enzymes following exercise.

Key Words: horse, selenoprotein, skeletal muscle oxidative damage

831 Fatty acid composition of synovial fluid in horses fed long chain polyunsaturated fatty acids: A pilot study. T. N. Ross*, T. M. Hess, J. D. Kisiday, C. W. McIlwraith, T. Engle, D. K. Hansen, J. Rexford, N. Schauermann, and C. Mulligan, *Colorado State University*, *Fort Collins*.

Studies utilizing oral α linolenic acid (ALA) and its metabolic derivatives, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), indicate a potential regulatory role in reducing joint inflammation by mediating cytokine production in arthritic human patients and plasma prostaglandin levels in arthritic horses. The primary study objective was to determine if dietary long chain fatty acids are incorporated into the synovial fluid of mature horses fed one of 3 dietary treatments. Twenty-one mature mixed breed mares, between 5 and 14 yr of age, with no history of joint disease and free of lameness were blocked by age, weight and body condition score and randomly assigned into one of 3 dietary treatment groups. Group 1 (FISH) received 69 mg/kg BW of n-3 EPA/DHA via a commercial fish oil supplement (Magnitude; JBS United, Sheridan, IN) daily; Group 2 (FLAX) received 68.6 mg/kg BW of n-3 ALA via a flaxseed supplement (Nutra-Flax) daily and Group 3 (CONT) did not receive n-3 supplementation. Total ration composition of n-3 fatty acids were approximately 143.5 mg/kg BW (FISH), 142.5 mg/kg BW (FLAX) and 78.7 mg/kg BW (CONT), respectively. On d 90, approximately 3 mL of synovial fluid was extracted from the right carpus of each horse and immediately placed into a 7 mL EDTA tube. Aliquots were assayed for fatty acid composition using gas chromatography. Data were analyzed using ANOVA, with significant differences ($P < 0.05$) further analyzed using Fisher's LSD test. Synovial fluid samples from the FISH group presented EPA and DHA in their synovial fluid, whereas the FLAX and CONT groups did not express detectable concentrations. The absence of EPA and DHA in the FLAX-fed group indicates inefficient conversion of these fatty acid metabolites from ALA. This is the first documented study evaluating synovial fluid fatty acid composition in horses receiving a dietary n-3 fatty acid supplement. Results indicate oral supplementation of EPA and DHA alters synovial fluid fatty acid composition. The presence of DHA and EPA in synovial fluid may potentially modify inflammatory processes in the joint.

Key Words: polyunsaturated fatty acids, horse, synovial fluid

832 Cushing's syndrome down-regulates glucose transporter mRNA abundance in the distal jejunum in the horse. A. Buckley, N. Taylor, R. Manjarin*, H. C. Schott, A. D. Woodward, and N. L. Trottier, *Michigan State University*, *East Lansing*.

Cushing's syndrome is commonly associated with the development of laminitis in the horse. High levels of glucocorticoids are known to induce insulin resistance and reduce cellular glucose uptake, however, the mechanisms are not known. Decreased capability for pre-cecal glucose absorption may increase carbohydrates flow to the large intestine in Cushing afflicted-horses, altering the hindgut microflora and increasing susceptibility to laminitis. The objective of this study was to test the hypothesis that horses affected by Cushing's Syndrome have lower mRNA abundance of genes encoding for glucose transporters and insulin receptor in the jejunum enterocytes, compared with healthy animals. Tissue from the distal jejunum was obtained from 8 adult horses (4 affected with Cushing's Syndrome and 4 healthy controls) shortly following euthanasia. The mucosa was gently separated from the sero-muscular layer, flash-frozen in liquid N, and stored at −80°C for RNA isolation. Gene expression of glucose transporters GLUT1 (SLC2A1), GLUT2 (SLC2A2), GLUT4 (SLC2A4), GLUT5 (SLC2A5), SGLT (SLC5A1) and insulin receptor (INSR) was assessed by measuring mRNA abundance using relative quantitative PCR. Succinate dehydrogenase complex subunit A (SDHA) and hypoxanthine phosphoribosyltransferase (HPRT) were used as reference genes in the study. Mixed model was used for data analysis. Compared with healthy control horses, mRNA abundance of insulin receptor and insulin-dependent glucose transporter GLUT4 decreased ($P < 0.01$) in distal jejunum of Cushing's horses. For GLUT1, GLUT2, GLUT5 and SGLT1, mRNA abundance did not differ between Cushing and control horses. Results indicate that Cushing's syndrome downregulates the gene expressions of insulin-dependent glucose transporter GLUT4 and insulin receptor in the distal jejunum of the horse. These results may offer a physiological mechanism for increased susceptibility to nutritionally induced laminitis in Cushing-afflicted horses.

Key Words: Cushing, horse, insulin

833 Proteomic analysis of synovial fluid and plasma from horses fed a high or low starch diet. E. A. Nowelsky*[1], J. K. Morrissey[1], D. S. Gibson[2], P. A. Harris[3], and W. B. Staniar[1], [1]*The Pennsylvania State University*, *University Park,* [2]*University of Colorado Denver*, *Aurora,* [3]*Equine Studies Group, Waltham Centre for Pet Nutrition*, *Melton Mowbray, UK*.

A tentative link has been suggested between osteochondrosis (OC) and feeding high starch diets in growing horses. The pathology of OC is multifactorial and it is therefore appropriate to explore using a technique that allows for characterization and quantification of the plasma (PL) and synovial fluid (SF) proteomes. The objective of this study was to determine if the concentration of dietary starch influences the proteomes of SF and PL and to isolate, identify and characterize proteins that are differentially present in relation to diet or sample type. Six yearlings, maintained on dry lot with ad libitum access to <5% starch hay, were used in this crossover study design conducted over 12 weeks with four 21-d periods. During periods 2 and 4, yearlings were split into 2 treatment groups, with one group receiving high starch feed (~40% starch on a DM basis)(HSF) and the other group receiving low starch feed (<10% starch on a DM basis)(LSF). All yearlings received LSF in periods 1 and 3. SF and PL samples were taken every 21 d and analyzed using 2D gel electrophoresis. Progenesis SameSpots software was used to analyze spot intensities using ANOVA and differences were considered significant at $P < 0.05$. Select spots were chosen for mass spectrometry. Changes in protein expression ranged from a 1.2 fold–2.5 fold change. Seven proteins were higher as a result of dietary treatment. Four of the 7 proteins, identified as isoforms of immunoglobulin gamma, were higher in SF from animals fed HSF. Two proteins, identified as isoforms of albumin, were higher in HSF samples. Fibrinogen, haptoglobin and clusterin were higher in PL. Alpha 1 antitrypsin was higher in SF. The grouping of isoforms and proteins with similar characteristics on the gel was useful in achieving the goals of this study. Proteins identified in this study initially do not have a clear mechanistic link with OC. This study demonstrates a novel approach to investigate protein expression changes in PL and SF that may affect joint health. The fluid specific proteomic profiles and individual proteins identified may be useful in future examination of equine biological fluids.

Key Words: equine, nutrition, proteomics

834 Effects of a 24-h feed withdrawal on SGLT1, GLUT5, and PepT1 gene expression in the small intestine and right ventral colon of the horse. B. E. Aldridge*, T. B. Lescun, and J. S. Radcliffe, *Purdue University*, *West Lafayette, IN*.

Fourteen horses were used to determine the effects of a 24 h feed withdrawal (FW) on gene expression in the small intestine and right ventral colon (RVC). Prior to FW, horses were fed hay ad libitum for 8 weeks. Following this adaptation period, horses were randomly allotted to either 24 or 0 h FW (7 horses/trt) with water provided ad libitum. Following 0 or 24 h of FW, horses were killed and segments of proximal jejunum (13.7 m proximal to the ileo-cecal junction), distal jejunum (9.1 m proximal to the ileo-cecal junction), ileum (5 cm proximal to the ileo-cecal junction) and right ventral colon (30 cm distal to the cecocolic junction) were removed for gene expression analysis of the sodium dependent glucose transporter (SGLT1), fructose transporter (GLUT5) and di- and tri-peptide transporter (PepT1) by using quantitative RT-PCR normalized to GAPDH. The GLM procedure in SAS was used to determine statistical differences between the intestinal sections of fed and non-fed horses, where horse served as the experimental unit. Horses maintained on a hay diet, had 82% more GLUT5 expression ($P < 0.05$) in the proximal jejunum (PJ), than horses subjected to a 24 h FW. However, in horses subjected to a 24 h FW, distal jejunal (DJ) SGLT1 expression increased ($P < 0.1$) 205% and PepT1 expression increased 288%, although this only approached a trend ($P = 0.13$). SGLT1, GLUT5 and PepT1 expression were not affected ($P > 0.1$) by FW in the ileum and right ventral colon. To compare gene expression across sections, the relative abundance was normalized to the distal jejunum. The PJ contained 13, 3.8 and 1.4- fold greater expression of SGLT1 ($P < 0.05$), GLUT5 ($P < 0.05$) and PepT1 ($P < 0.05$) compared with the DJ. Expression of SGLT1, GLUT5 and PepT1 were 34, 19 and 15% lower in the ileum than in the DJ, and 91, 63 and 84% lower in the RVC compared with the DJ. This data indicates that SGLT1, GLUT5 and PepT1 are expressed throughout the small intestine and in the RVC of the horse at varying concentrations, and that they are differentially regulated by a 24-h feed withdrawal.

Key Words: horse, gene expression, intestine

835 Effects of omega-3 fatty acid supplementation on plasma, red blood cell and muscle cell fatty acid compositions in horses. J. K. Rexford*, T. M. Hess, N. L. Schauermann, T. E. Engle, D. K. Hansen, K. D. Allen, and C. M. Mulligan, *Colorado State University*, *Fort Collins*.

The objective of this study was to examine the effects of dietary omega-3 fatty acid supplementation on plasma, red blood cell and muscle omega-3

fatty acid compositions in horses. Twenty-one mares were blocked by age, body weight and body condition score and randomly assigned to one of 3 dietary treatments. Treatments consisted of: 1) 142.5 mg/kg BW of n-3 fatty acids via a fish oil supplement and diet (FISH; Magnitude; JBS United, Sheridan, IN); 2) 142.5 mg/kg BW of n-3 fatty acids via a flaxseed meal from the supplement and diet (FLAX; Nutra-Flax^{TM}); and 3) control (CON) no supplemental fatty acid with 78.7 mg/kg BW of omega-3 fatty acids from the diet (mostly hay for all groups). Treatments were supplemented for 90 d. Blood samples and muscle middle gluteal biopsies were taken on d 0, 30, 60, and 90 of supplementation. Plasma and cell fatty acid profiles were analyzed via gas chromatography. Plasma linoleic acid (LA) and α linolenic acid (ALA) were 10 and 40% lower ($P < 0.008$) respectively in the FISH compared with FLAX and CON supplemented horses. Plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were only detected in the FISH supplemented horses and increased 29% ($P < 0.001$) and 17% ($P < 0.005$) from d 30 to d 60. Red blood cell LA and ALA were not different between treatments. Red blood cell EPA and DHA were only detected in the FISH supplemented horses, where EPA increased 4% ($P < 0.005$) from d 30 to d 60. Red blood cell DHA increased 36% ($P < 0.004$) between d 30 and d 90. Muscle LA was 15% lower in the FISH supplemented horses compared with the other treatments. Muscle ALA was 42% lower ($P < 0.030$) in the FISH supplemented horses, compared with FLAX and CON groups. Muscle EPA was 32% higher ($P < 0.001$) in FISH supplemented horses compared with other treatments and increased by 40% from d 30 to d 60. Muscle DHA was 43% higher ($P < 0.001$) in the FISH supplemented horses compared with other treatments and increased by 38% between d 30 and 90. This is the first study to demonstrate that dietary fatty acid supplementation will affect muscle fatty acid composition in horses.

Key Words: equine, eicosapentaenoic acid, docosahexaenoic acid