

Horse Species I

360 Influence of maternal plane of nutrition and arginine supplementation on mares and their foals: Determination of voluntary dry matter intake of mares during late pregnancy. K. N. Winsco*¹, J. A. Coverdale¹, T. A. Wickersham¹, C. J. Hammer^{2,3}, and J. L. Lucia¹, ¹Department of Animal Science, Texas A&M University, College Station, ²Department of Animal Sciences, North Dakota State University, Fargo, ³Center for Nutrition and Pregnancy, North Dakota State University, Fargo.

Thirty-two mares (468–668 kg BW; 3–19 yr) were blocked by expected foaling date and randomly assigned within block to treatments. Treatments were arranged as a 2 × 2 factorial with 2 planes of nutrition (Nutr), moderate (Mod; 0.5% BW AF grain/d) or high (High; 1% BW AF grain/d) and 2 levels of L-arginine supplementation, 0.21 g/kg/d (Arg) or no supplemental Arg (Con; L-alanine to maintain isonitrogenous diets). The objective was to evaluate the impact of altered plane of nutrition on voluntary forage dry matter intake (FDMI) and determine the ability of Arg to mitigate these effects. Treatments began 110 d before expected foaling date and terminated at parturition. Mares were housed by block and allowed ad libitum access to coastal bermudagrass (*C. dactylon*) hay, and fed commercial grain 2 × /d in individual stalls. To evaluate FDMI, a dual marker system was used at 9, 10, and 11 mo gestation. Titanium dioxide (TiO₂) was dosed at 10 g/d for 14 d by top dressing onto the grain meal. Fecal grab samples were obtained the last 4 d of TiO₂ supplementation 2 × /d via rectal palpation at 12 h intervals with times advancing 3 h each d to account for diurnal variation. Fecal samples were analyzed for TiO₂ using a colorimetric procedure (Titgmeier et al., 2001). Fecal, grain, and hay samples were analyzed for ADIA using the ANKOM fiber system. Data were analyzed using the PROC MIXED procedure of SAS. There was no effect of Arg on FDMI ($P \geq 0.60$). Nutrition tended to influence FDMI ($P \leq 0.10$) with Mod mares consuming a greater percentage of their BW compared with High. Regardless of dietary treatment, month of gestation influenced FDMI ($P \leq 0.01$) with all mares consuming less during the 11th mo ($P \leq 0.05$). Upon calculation, grain contained 3.60 Mcal/kg DE and hay contained 1.98 Mcal/kg. Based on calculated FDMI, Mod mares consumed an average of 28.21 Mcal DE/d while High mares consumed 34.60 Mcal DE/d which exceed NRC (2007) requirements for late gestation. In summary, maternal plane of nutrition had a tendency to alter FDMI and FDMI was influenced by gestation.

Key Words: intake, arginine, broodmares

361 The effect of hay steaming on forage quality and intake by horses. J. E. Earing,* M. R. Hathaway, C. C. Shaeffer, J. C. Paulson, S. L. Privatsky, and K. L. Martinson, University of Minnesota, St. Paul.

Heaves is a common equine disease. Current management strategies include soaking or wetting hay before feeding. Hay steaming is gaining popularity in the US, however, little is known about its impact on forage quality or palatability. Therefore, the objectives were to determine the effect of steaming on forage quality and intake. Two alfalfa-orchardgrass hays were tested: a moderately moldy hay (MM) and a lower mold hay (LM). While nutrient composition was similar, the mold content differed (MM: 373,000 cfu/g; LM: 120,500 cfu/g; $P = 0.0003$). Six mature horses were used in a 10 d crossover design. Three horses were assigned to each hay type; treatments were switched on d 6. Each day, one bale of each hay was steamed for 90 min using a commercial hay steamer. Two flakes of steamed and 2 flakes of unsteamed hay (MM or LM) were weighed

and offered simultaneously to each horse in individual hay nets. Hay nets were located on opposite walls of the stall; location of the steamed and unsteamed hay was switched daily. The amount of hay offered was in excess of ad libitum intake. Horses were allowed access to hay for 2 h starting at 15:00 h, then orts were collected and DMI calculated. For each hay, paired *t*-tests were used to compare steamed and unsteamed hay nutrient content and DMI. Prior to steaming, the DM of both hays was similar (90%); steaming significantly reduced DM to 81 and 77% for MM and LM, respectively. In both MM and LM, steaming reduced P content ($P < 0.007$). Steaming reduced WSC by 13% ($P = 0.001$) and ESC by 27% ($P = 0.003$) for MM, but had no effect on LM ($P > 0.05$). Similarly, steaming reduced mold levels in MM by 85% ($P = 0.009$), but did not affect levels in LM ($P > 0.05$). No other forage quality components were affected by steaming. DMI of MM was not affected by steaming ($P > 0.05$); intake averaged 1.34 kg of unsteamed and 1.21 kg of steamed hay. Intake of LM was affected by steaming; horses ingested 0.64 kg of unsteamed and 2.02 kg of steamed hay ($P < 0.0001$). In moderately moldy hay, steaming reduced mold levels, but did not improve intake. However, for hay with low mold levels, steaming appeared to increase the palatability of the hay, while exerting no effects on forage quality.

Key Words: DMI, equine, mold

362 High non-structural carbohydrate diet in ponies alters location and absorptive capacity of glucose, phosphorus and glutamine across the gastrointestinal tract. B. E. Aldridge*¹, A. D. Woodward², J. S. Radcliffe¹, R. J. Geor³, L. J. McCutcheon³, and N. L. Trottier², ¹Purdue University, West Lafayette, IN, ²Michigan State University, Department of Animal Science, East Lansing, ³Michigan State University, Department of Large Animal Clinical Science, East Lansing.

Sixteen ponies were used to test the hypothesis that exposure to a rich non-structural carbohydrate (NSC) containing diet decreases small intestinal (SI) glucose transport capacity, and affects large intestinal (LI) absorptive nutrient profile compared with a strict forage-based diet. Ponies ($n = 16$) of mature age (BW 270 ± 74.4 kg) were equally assigned and fed one of 2 diets ($n = 8$) containing 12% CP: a control diet (CON) composed of grass hay (2.3% BW) and balancer pellet (0.2% BW), and a high NSC diet (CHO) containing 42% NSC, and composed of sweet feed and oligofructose (1.5% BW), grass hay (0.95% BW) and balancer pellet (0.05% BW). Ponies were subjected to a 4-wk adaptation period to the CON diet, followed by individual feeding of test diets for 7 d and euthanasia by single injection of pentobarbital. Segments of jejunum (J), ileum (I), left ventral (LVC) and left dorsal colon (LDC) were immediately mounted in Ussing Chambers to measure capacity for active glucose, phosphorus (P), and glutamine (Gln) absorption, based on change in short circuit current (I_{sc} , $\mu A/cm^2$). Feeding CHO decreased active glucose transport ($P < 0.001$) across J and I compared with CON. Glucose transport across LVC and LDC did not differ between diets. In ponies fed CON, glucose transport capacity increased ($P = 0.008$) across J and I compared with that of the LVC and LDC, but in ponies fed CHO, glucose transport capacity between small and large intestinal segments did not differ. Feeding CHO did not affect active transport capacity of Gln or P across J and I, and increased ($P = 0.048$) active transport capacity of Gln by 239 and 60% and of P by 52.7 and 30.7% across LVC and LDC, respectively. Carbachol-induced Cl ion secretion was 30% lower ($P = 0.048$) across intestinal sections in CHO-fed

ponies. In conclusion, abrupt exposure to NSC decreased SI glucose active transport capacity, increased LI active transport capacity of P and Gln, and reduced Cl ion secretion. Results indicate that abrupt dietary intake of NSC reduces the absorption of glucose by the SI of equids.

Key Words: equine, carbohydrate, intestine

363 Exercise-induced suppression of lymphocyte function is unaffected by a higher level of dietary selenium. J. Bobel,* L. K. Warren, and S. White, *University of Florida, Gainesville.*

The effect of dietary selenium (Se) concentration on lymphocyte function and viability following prolonged exercise was evaluated in 12 unconditioned Thoroughbred horses (mean \pm SE, 11 \pm 1 y, 565 \pm 11 kg). Horses were randomly assigned to receive either 0.1 mg Se/kg DM (NRC-Se; n = 6) or 0.3 mg Se/kg DM (HIGH-Se; n = 6) for 36 d. Horses were individually fed 1.6% BW/d of coastal bermudagrass hay (0.02 mg Se/kg), 0.4% BW/d of whole oats (0.24 mg Se/kg) 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 (26 km) of submaximal exercise in a free-stall exerciser (heart rate 135 \pm 39 bpm). Blood samples were obtained on d 0 and 34 for determination of serum Se, and before exercise and at 6 and 24 h post-exercise for identification of leukocyte populations and isolation of peripheral blood mononuclear cells. Data were analyzed using the MIXED procedure of SAS (v. 9.2) with repeated measures. Serum Se remained unchanged in NRC-Se horses, but increased ($P < 0.01$) in HIGH-Se horses after 34 d of supplementation. Exercise resulted in an increase in circulating neutrophils ($P < 0.001$) and decreases in lymphocytes ($P < 0.001$) and eosinophils ($P < 0.001$), which persisted through 24 h post-exercise. Suppressed lymphoproliferative responses to concanavalin A ($P < 0.001$), phytohemagglutinin ($P < 0.01$) and pokeweed ($P < 0.01$) mitogens were noted at 6 h and 24 h post-exercise. Lymphocyte cell viability following in vitro hydrogen peroxide exposure was decreased ($P < 0.05$) at 24 h post-exercise. Level of dietary Se had no effect on leukocyte populations or lymphocyte proliferation or viability following exercise. These data indicate lymphocytes may be more vulnerable to oxidative damage and may not function properly during recovery from exercise, which could put horses at risk for infection. Feeding Se at 3 \times the current NRC recommendation failed to mitigate exercise-induced suppression of lymphocyte viability and function.

Key Words: sodium selenite, prolonged exercise, immune function

364 Feeding graded amounts of lysine to yearling Thoroughbred colts does not activate the mTOR signaling pathway. S. L. Tanner,* L. R. Good, E. A. DeLuca, R. J. Coleman, and K. L. Urschel, *University of Kentucky.*

Lysine (Lys) is known to be the first limiting amino acid (AA) in typical equine diets and therefore it is of prime interest when considering the nutrition of growing horses. AAs, as a group, have been shown to stimulate the mammalian target of rapamycin (mTOR) pathway and the activation of this pathway has been used as an indicator of protein synthesis at the tissue level, particularly in muscle. However, it is unknown whether Lys intake specifically can affect the activation of mTOR signaling factors. The objective of this study was to determine how feeding graded amounts of Lys affects mTOR signaling activation in growing horses. Six Thoroughbred colts (401 \pm 5 d) were studied while receiving each of 6 levels of Lys intake: 80, 95, 110, 125, 135, and 145 mg/kg/d in a 6 \times 6 Latin square. Diets consisted of a concentrate portion, which varied in Lys, and timothy hay cubes. Diets were isocaloric, isonitrogenous,

and met or exceeded NRC recommendations for all nutrients except lysine. Each horse was fed each diet in random order for a period of 6 d. On d 6 a venipuncture sample and gluteal medius muscle biopsy were collected approximately 100 min post feeding of the morning meal. The ratio of phosphorylated to total protein was determined via Western blots quantified using ImageJ. Although plasma Lys concentrations increased in a dose-dependent manner with increasing dietary Lys intake ($P < 0.0001$), Lys intake did not affect eukaryotic initiation factor 4E binding protein 1 (4EBP1), ribosomal protein S6 (rps6), or protein kinase B (Akt) ($P > 0.1$) activation. Despite differences in Lys intake, the diets did provide the same amount of nitrogen and therefore these results suggest that Lys is not an independent activator of mTOR signaling. Alternatively, it is also possible that mTOR signaling was already maximized by the lowest intake of Lys provided and that subsequent increases in Lys intake did not result in any further increases in mTOR signaling. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2010-65206-20638 from the USDA National Institute of Food and Agriculture.

Key Words: lysine, horse, mTOR signaling

365 Influence of oral glucosamine supplementation on young horses in training: Pharmacokinetics. J. L. Lucia*¹, K. L. Gehl¹, J. A. Coverdale¹, C. E. Arnold², R. Dabareiner², and E. D. Lamprecht³, ¹*Department of Animal Science, Texas A&M University, College Station* ²*Large Animal Teaching Hospital, Texas A&M University, College Station*, ³*Cargill Animal Nutrition, Elk River, MN.*

Twenty-one yearling Quarter horses (442 to 565 d of age and 351 to 470 kg) were utilized in a pilot trial to determine pharmacokinetic parameters of oral glucosamine derived from a non-GMO fungal biomass fermentation product. Horses were arranged in a randomized complete block design, and assigned 1 of 3 treatments via nasogastric intubation: control (CON; no glucosamine, 1000 mL saline), glucosamine HCl 15 mg/kg BW (GLU15; Activesure liquid; Cargill, Eddyville, IA), and glucosamine HCl 30 mg/kg BW (GLU30; Regenasure powder; Cargill). Horses were fasted for 12 h before nasogastric intubation. The GLU15 and GLU30 treatments were diluted in saline to reach a volume of 500 mL. Horses receiving GLU15 and GLU30 were given a saline flush of 500 mL post administration to ensure treatment was received in its entirety and reach a final volume of 1000 mL. Blood samples were obtained via jugular venipuncture at 0, 1.5, 3, and 12 h after treatments were administered. Plasma was harvested and stored at -20°C , before glucosamine analysis via HPLC. Data were analyzed using PROC MIX procedure of SAS. Plasma glucosamine concentrations increased as dosage of oral glucosamine increased ($P \leq 0.01$). Following intubation, plasma glucosamine values for GLU15 and GLU30 peaked at 3.0 h ($P \leq 0.05$) and 1.5 h ($P \leq 0.01$), respectively, and returned to baseline by 12 h. Additionally, there was a treatment by time interaction with GLU30 horses experiencing a sharp rise in plasma glucosamine concentration by 1.5 h post administration, resulting in a 6-fold increase compared with horses receiving GLU15 ($P \leq 0.01$). Both glucosamine products resulted in increased plasma glucosamine concentrations and the response was dose dependent. Further research will be conducted to determine the influence of oral glucosamine supplementation over time, including plasma and synovial fluid incorporation.

Key Words: equine, glucosamine, pharmacokinetics

366 Effects of prolonged exercise and citrulline supplementation on metabolic status in equine blood and skeletal muscle. S. White,* L. K. Warren, S. E. Johnson, and B. Miller, *University of Florida, Gainesville.*

Metabolic gene expression in skeletal muscle, as well as indicators of oxidative stress, in response to prolonged exercise and citrulline supplementation were evaluated in 12 untrained Thoroughbred and Quarter Horse mares (mean \pm SE 11 \pm 0.7 y, 552 \pm 9 kg). Horses were randomly assigned to one of 2 isonitrogenous supplements for 15 d: 86 mg citrulline malate/kg BW (CIT; n = 6) or 25 mg urea/kg BW (n = 6). Additionally, horses were individually fed a fortified commercial feed and Coastal bermudagrass hay. On d 15, horses underwent 2 h (27.5 km) submaximal exercise in a free-stall exerciser (mean heart rate 142 \pm 2 bpm). Blood samples were obtained before exercise and 1 h post-exercise for analysis of serum creatine kinase (CK) activity and total plasma lipid hydroperoxides (LPO). Biopsies of the middle gluteal muscle were obtained before exercise and 1 h post-exercise and gene expression was determined by quantitative RT-PCR using 18S as a reference gene. Data were analyzed using the MIXED procedure of SAS (v. 9.2) with repeated measures. Muscle expression of *MT1B* increased ($P = 0.04$) nearly 2.5-fold from pre- to 1 h post-exercise, indicating the exercise was sufficient to elicit oxidative stress. Muscle expression of *PDK4* increased ($P = 0.002$) over 64-fold from pre- to 1 h post-exercise, whereas expression of *iNOS*, *GLUT4*, *PGC1 α* , and *SLC7A* remained unchanged through 1 h post-exercise. Serum CK activity ($P = 0.009$) and plasma LPO ($P = 0.04$) increased from pre- to 1 h post-exercise. However, CIT supplementation had no effect on expression of *MT1B*, *PDK4*, *iNOS*, *GLUT4*, *PGC1 α* , or *SLC7A* in muscle or on serum CK activity or plasma LPO following exercise. Prolonged exercise in untrained horses upregulates aerobic metabolism (via expression of *PDK4*) and also appears to result in measurable tissue damage and lipid peroxidation. However, short-term CIT supplementation at rates sufficient to decrease production of reactive oxygen species in human athletes had no effect on markers of oxidative stress in exercised horses.

Key Words: horse, skeletal muscle oxidative damage, skeletal muscle metabolism

367 Selenium supplementation and immune function. M. Brummer,* S. Hayes, A. Betancourt, A. A. Adams, D. W. Horohov, and L. M. Lawrence, *University of Kentucky, Lexington.*

Some studies indicate an effect of Se status on immune function. This study investigated the effect of Se supplementation on Se status and immune response in mature horses. Twenty-eight horses, blocked by age and sex, were randomly allocated to one of 4 groups: LS, AS, SP and SS. For 35 wk LS, SP and SS received a low Se diet (0.07 ppm Se) and AS received an adequate Se diet (0.14 ppm Se). For the next 29 wks LS and AS were maintained on the same diet while SP received a high organic Se diet (0.3 ppm; Sel-Plex, Alltech, Nicholasville, KY) and SS received a high inorganic Se diet (0.3 ppm; sodium selenite). The basal diet consisted of low Se pasture, hay, cracked corn and a balancer pellet either low (LS, SP, SS) or adequate (AS) in Se. The SP and SS supplements were top dressed on the balancer pellet. Whole blood Se and glutathione peroxidase activity (GPx) were monitored monthly. Horses were vaccinated with 10 mg ovalbumin (OVA) at wk 22 and 25. Blood samples were taken for 7 wk following vaccination for serum separation and at pre-, 3 and 5 wk post initial vaccination for peripheral blood mononuclear cell (PBMC) isolation, and whole

blood cytokine mRNA evaluation. Data were analyzed as ANOVA with repeated measures (SAS 9.2). An effect of treatment, time and treatment x time ($P < 0.05$) existed for both Se and GPx. At the start of the 29 wk period Se was lower for LS, SP, and SS compared with AS. At 22 wk Se was higher for SP and SS than AS ($P < 0.05$). Whole blood GPx had a similar but delayed response. At 27 wk GPx was higher for SP and SS than AS, suggesting that maximum GPx activity is not maintained by dietary Se intakes of 0.1 ppm. The response to OVA vaccination, evaluated as OVA specific IgG production, cytokine mRNA expression of PBMC stimulated with OVA in vitro, and lymphocyte proliferation was unaffected by Se status. However, PBMC stimulated with phorbol 12-myristate 13-acetate indicated lower mRNA expression of some cytokines for LS ($P < 0.05$). Whole blood mRNA expression of IL-10 was higher for SS compared with LS, AS and SP ($P < 0.05$). Although the OVA vaccination response was unaffected by Se status, other measures of immune function suggest that low Se status affects cell-mediated immunity.

Key Words: horse, glutathione peroxidase, ovalbumin

368 Effect of strenuous exercise on stallion sperm quality. J. L. Rosenberg,* C. A. Cavinder, C. C. Love, M. M. Vogelsang, S. R. Teague, D. H. Sigler, D. D. Varner, and T. L. Blanchard, *Texas A&M University, College Station.*

Performance ability of an equine athlete is one of the major considerations when selecting a stallion for breeding, and thermal stress associated with training exercise may affect sperm quality and the future reproductive capability of the stallion. Previous investigators reported that a prolonged increase in testicular temperature (3–3.5°C) decreased percentages of progressive motility and morphologically normal sperm. A subsequent study found contrasting results with no effect on semen quality when stallions were subjected to moderate exercise (0.8°C increase in internal scrotal temperature). It remains unclear what level of exercise imparts a negative effect on sperm quality. The objectives of this study were to compare sperm quality of strenuously exercised (EX) and non-exercised (CN) stallions. Strenuous exercise was determined by maintaining a heart rate between 145 and 155 bpm during work bouts. Miniature stallions (n = 7) implanted with subdermal thermosensory devices in the subcutaneous neck and scrotal tissue were matched by age and sperm quality and assigned to treatment group. Stallions (EX; n = 3) were exercised 4 d/wk for 90 min for 12 wk. Semen was collected from stallions for 5 consecutive d every 4 wks to evaluate semen quality (raw, 24 h and 48 h cooled). Internal scrotal (ST), rectal (RT) and neck (NT) temperatures were recorded along with heart rate of the stallions. Spermatozoa data were normally distributed; therefore, they were subjected to parametric analysis by repeated measures (wk) using the PROC MIXED procedure (SAS v 9.1; SAS Inst. Inc., Cary, NC). Model included treatment (CN or EX), time (wk 0, 4, 8, or 12), and stallion as the subject of the repeated measures. Compared with the CN group, EX stallions had elevated body temperature (avg RT 39.0 vs 37.2°C, NT 40.9 vs 38.5°C, and ST 34.9 vs 33.4°C) but parameters of spermatozoa quality remained unaffected by treatment ($P > 0.05$). While previous studies have illustrated that prolonged insulation of the testes reduces semen quality, strenuously exercising stallions for up to 90 min under hot and humid ambient conditions may not be harmful to spermatogenesis.

Key Words: heat stress, sperm, stallion