

Horse Species

TH266 Effects of melatonin on stallion sperm motility and viability in vitro. A. Trabold¹, J. M. Reddish¹, K. Barnhart¹, M. A. Coutinho da Silva², and K. Cole^{*1}, ¹Department of Animal Sciences, The Ohio State University, Columbus, ²Department of Veterinary Clinical Sciences, The Ohio State University, Columbus.

Recent research has shown that melatonin has positive effects on sperm viability during cryopreservation. Therefore, melatonin could potentially be included in commercial stallion semen extender to aid in the survival and function of fresh cooled spermatozoa. The objective of this study was to determine effects of melatonin on stallion sperm motility and viability during 48 h of storage at 5°C. In 4 separate trials, ejaculates from 3 stallions were collected and diluted to a final concentration of 500 million sperm/ejaculate with a skim milk-based extender, without antibiotics, supplemented with 0, 0.1, 1.0 or 10.0 mM of melatonin and stored at 5°C for 48 h. Total motility (TM), progressive motility (PM), track velocity (VCL), straight line velocity (VSL) and smoothed path velocity (VAP) were evaluated by Computer-Assisted Semen Analysis (CASA) at 0, 24 and 48 h of storage. An eosin-nigrosin stain was used to subjectively evaluate the live/dead ratio of spermatozoa at 0, 24 and 48 h of storage. Data were analyzed using the PROC MIXED procedure of SAS. TM, PM and sperm viability decreased over time ($P < 0.05$); however, there were no differences in TM and PM due to melatonin concentrations at any time point. At 48 h, the addition of 10 mM of melatonin to the extended semen had significantly higher PM ($P < 0.05$). At 24 and 48 h, semen extended with 10mM of melatonin had significantly decreased VCL and VAP compared with control ($P < 0.05$). VSL and VAP decreased from 0 to 24 h, but there were no differences in VSL between 24 and 48 h. Overall, addition of melatonin to stallion semen extender did not affect sperm motility and viability during storage at 5°C in this study.

Key Words: stallion, semen, melatonin

TH267 Influence of prebiotic and probiotic supplementation on apparent digestibility in mature geldings at maintenance. J. A. Coverdale^{*1}, E. D. Lamprecht², P. Kropp³, I. Yoon³, J. L. Lucia¹, K. N. Winsco¹, A. E. Hanson¹, and C. M. Warzecha¹, ¹Texas A&M University, College Station, ²Cargill Incorporated, Elk River, MN, ³Diamond V, Cedar Rapids, IA.

Twenty-seven mature Quarter horse geldings (465 to 663 kg BW and 4 to 24 yr old) were utilized in a randomized complete block design to evaluate effect of prebiotic and probiotic dietary fortification on apparent digestibility. Horses were blocked by age and BW and randomly assigned to treatment within block for a 28-d trial. Dietary treatments consisted of whole oats (Oat), an unfortified pellet (Control) without pre- or probiotics and inorganic trace minerals (Cargill, Inc., Elk River, MN), or the same pellet (Fortified) including a prebiotic (14 g per d *Saccharomyces cerevisiae* yeast fermentation culture dehydrated, commercial name Original XPC, Diamond V, Cedar Rapids, IA), probiotic (min 2.3 million cfu/kg feed each of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Enterococcus faecium* fermentation products dehydrated), and complexed trace minerals. Dietary treatments were offered individually at 0.5% BW (as fed) in addition to 1.5% BW (as fed) of coastal bermudagrass hay (*Cynodon dactylon*) per d. Physical measurements were recorded every 7 d. The final 4 d consisted of total fecal collections. Concentrate, hay, and fecal samples were analyzed for nutrient composition and apparent digestibility determined. Data were

analyzed using the PROC MIXED. Treatment means were compared using pre-planned orthogonal contrasts: pelleted concentrate vs. oats (Control + Fortified vs. Oat) and Control vs. Fortified. Rump fat, BW, and BCS were unaffected by dietary treatment. Apparent digestibility of DM, OM, CP, and gross energy was also not influenced by diet. Apparent digestibility of NDF and ADF was greater ($P = 0.03$) for horses fed pellets compared with Oat. Between pelleted concentrates, digestibility of NDF and ADF tended ($P = 0.13$) to be greater for horses fed Fortified compared with Control. In conclusion, digestibility of ADF and NDF is improved when pelleted diets are fed to mature horses and further improved with addition of prebiotic, probiotic, and complexed trace mineral preparations.

Key Words: digestibility, prebiotic, probiotic

TH268 Influence of oral glucosamine supplementation in young horses: Dietary adaptation. J. L. Lucia^{*1}, K. L. Gehl¹, J. A. Coverdale¹, C. E. Arnold¹, R. A. Dabareiner¹, K. N. Winsco¹, and E. D. Lamprecht², ¹Texas A&M University, College Station, ²Cargill Incorporated, Elk River, MN.

Fourteen yearling Quarter horses (351 to 470 kg) were utilized in a randomized complete block design to evaluate absorption of supplemental dietary glucosamine hydrochloride (HCl) and incorporation into plasma and synovial fluid over 98-d. Horses were blocked by BW, age, and sex, and randomly assigned to treatment within block. Dietary treatments included control (no glucosamine HCl; CON) or 30 mg/kg BW/d glucosamine hydrochloride (HCl) powder derived from a non-GMO fungal biomass fermentation product (GLU30; 99.6% purity Regensure powder; Cargill, Inc., Eddyville, IA). Diets consisted of CON horses (n = 7) fed 1% BW (as-fed) concentrate only or GLU30 horses fed (n = 7) the same concentrate with additional GLU30 offered at 12 h intervals. Horses were maintained in individual stalls and offered approximately 1% BW per d of coastal bermudagrass hay (*Cynodon dactylon*). Plasma and synovial fluid samples were obtained every 14 and 28 d, respectively, and stored at -20°C, before analysis of glucosamine via HPLC. Synovial samples were obtained from both radiocarpal joints and pooled. Data were analyzed using PROC MIXED procedure of SAS. Plasma and synovial glucosamine concentrations tended ($P = 0.10$ and $P = 0.06$, respectively) to increase in response to GLU30 compared with CON. There was also a treatment by time interaction ($P \leq 0.01$), with GLU30 increasing plasma glucosamine concentrations at 28 and 42 d when compared with CON. Similar to plasma, a treatment by time interaction ($P \leq 0.01$) was observed with GLU30 increasing synovial glucosamine levels at d 28 and 84 ($P \leq 0.01$ and $P = 0.05$, respectively). These results indicate that an oral glucosamine supplement is successfully absorbed and results in increased plasma and synovial fluid concentrations of glucosamine when fed to young horses.

Key Words: glucosamine, horse, synovial fluid

TH269 The availability of dietary calcium and magnesium to long yearlings and mature horses. A. L. Fowler^{*}, L. A. Strasinger, T. L. Hansen, B. E. Harlow, S. H. Hayes, and L. M. Lawrence, University of Kentucky, Lexington.

When formulating requirements, the NRC (2007) assumes that growing and adult horses have the same ability to digest Ca and Mg. However, very few studies have compared the digestibilities of dietary minerals

by different aged horses. This study examined Ca and Mg digestibility of a forage:concentrate diet fed to long yearlings and adult horses. Four long yearling geldings (19 ± 1 mo; 478 ± 58.9 kg) and 4 mature geldings (10.5 ± 7.5 yr; 541 ± 45.9 kg) were randomly divided into 2 separately studied blocks, each consisting of 2 long yearlings and 2 adult horses. Horses were fed a diet of timothy cubes, alfalfa cubes and a pelleted concentrate. The diet contained 0.73% Ca and 0.25% Mg (dry matter basis). During a 14-d adaptation period, feed intake was adjusted to minimize orts and horses were accustomed to wearing fecal collection harnesses. Then for a 4-d period, total fecal output was collected from each horse. Fecal and feed samples were analyzed for Ca and Mg using atomic absorption and the results were used to calculate Ca and Mg digestibility. The effect of age on Ca and Mg digestibility was evaluated using GLM procedures (SAS 9.2). Apparent Ca digestibility tended to be lower ($P = 0.053$) for the long yearlings ($37.16 \pm 2.0\%$) than for the adult horses ($42.78 \pm 2.1\%$). Apparent Mg digestibility was not different between the 2 ages ($P > 0.05$) and averaged $39.79 \pm 3.2\%$ across the 2 groups. Endogenous fecal losses of Ca and Mg were calculated for mature and growing horses using values reported in NRC (2007) and used to estimate true digestibility. True Ca digestibility tended to be higher ($P = 0.058$) for the long yearlings ($78.71 \pm 1.2\%$) than for the mature horses ($70.57 \pm 3.0\%$). True Mg digestibility was not different between the ages ($P > 0.05$) and averaged $59.19 \pm 3.2\%$ across the 2 groups. The NRC (2007) estimates true Ca digestibility at 50% and true Mg digestibility at 40%. These results suggest that true Ca and Mg digestibility values used by NRC (2007) to calculate Ca and Mg requirements may be underestimated.

Key Words: equine, mineral, requirement

TH270 Changes in fecal microbial species richness during foal heat diarrhea. L. A. Strasinger^{*1}, A. L. Fowler¹, G. L. Gellin², M. D. Flythe^{2,1}, and L. M. Lawrence¹, ¹University of Kentucky, Lexington, ²USDA, ARS, Lexington, KY.

Foal heat diarrhea affects many newborn foals and typically occurs between 5 and 15 d post-foaling. While this phenomenon is named for its occurrence at the time the mare has her first postpartum estrus, the actual cause is still undetermined. One theory is that foal heat diarrhea is caused by the normal colonization of the neonatal gastrointestinal tract by bacteria. We hypothesized that the fecal microbial species richness in foals experiencing foal heat diarrhea would be greater than in the same foals before diarrhea. Fecal samples were collected from foals ($n = 6$) 4 d post-foaling and on the first d that foal heat diarrhea was observed. Samples were frozen at -20°C immediately after collection for later analysis using PCR-denaturing gradient gel electrophoresis (PCR-DGGE). Fecal consistency was scored on a scale from 0 to 3 (0 = normal feces, 1 = soft stool, 2 = particulate bundles in a liquid base, and 3 = mostly liquid with very little particulate matter present). Foals with fecal scores above 0 were considered to have foal heat diarrhea. Microbial DNA was extracted from each fecal sample and the small ribosomal subunit (16s rRNA gene) was amplified, then subjected to DGGE using a 40–60% gradient. Comparisons of the foal fecal microbial species richness were made by enumerating bands on gels from fecal samples collected on d 4 (no foal heat diarrhea) and on the first day of foal heat diarrhea. Body weights were measured on 0, 1, 2, 3, 4, 7, 9, 11, 17, 21, and 28 d post-foaling. The initiation of foal heat diarrhea ranged from 7 to 11 d post-foaling (9.7 ± 1.6 ; mean \pm SD) and lasted 4 ± 1.8 d. All foals had fecal scores of 3 on the first day of foal heat diarrhea. The average daily gain (ADG) for foals before the onset of foal heat diarrhea was 2.4 ± 0.6 kg/d but decreased ($P < 0.05$) to 1.1 ± 0.4 during foal heat diarrhea. Foal band count was 22.6 ± 2.4 (mean \pm

SE) before foal heat diarrhea however, during foal heat diarrhea band count increased ($P < 0.05$) to 32.6 ± 4.3 . While foal heat diarrhea is not considered infectious, these observations suggest that this phenomenon does affect ADG and the microbial community of the hindgut.

Key Words: equine, microbial ecology, bacterial flora

TH271 Equine palmar artery, palmar vein and uterine artery express different populations of vasoactive biogenic amine receptors. D. A. Hestad¹, K. J. McDowell¹, and J. L. Klotz^{*2}, ¹Department of Veterinary Science, University of Kentucky, Lexington, ²USDA-ARS FAPRU, Lexington, KY.

Consumption of endophyte-infected (*Neotyphodium coenophialum*) tall fescue (*Lolium arundinaceum*) seed by horses causes constriction of the palmar artery (PA), palmar vein (PV) and reduced blood flow to the corpus luteum that can be measured in vivo by Doppler ultrasonography. In addition, myograph studies demonstrated that some ergot alkaloids, as well as the biogenic amines norepinephrine and 5-hydroxytryptamine, cause constriction of the PA and PV in vitro, but that the PA and PV have different contractile responses to different alkaloids. The objective of this study was to characterize biogenic amine receptors in the PA, PV, and uterine artery (UA) of horses. Immediately after euthanasia, PA, PV and UA were collected from 14 mixed breed mares. In experiment 1, transcripts of the PA, PV and UA were analyzed by PCR for presence/absence of adrenergic (ADR), serotonergic (5-HTR), and dopaminergic (DRD) receptor mRNAs ($n = 4, 6,$ and 5 receptor subtypes, respectively). Of those, 2 ADR, 5 5-HTR, and 2 DRD receptor subtype mRNAs were consistently expressed. In experiment 2, relative abundance of 5-HTR subtype 1B, 1D, 2A, 2B and 7 mRNAs, as well as GAPDH mRNA, were determined using semiquantitative real-time PCR. Quantitation of mRNA expression was conducted using the relative standard curve method with expression normalized to GAPDH mRNA. Expression of receptor types differed across vessels ($P < 0.0001$). Within the PA, 5-HTR2B was more highly expressed ($P = 0.03$), while other subtypes did not differ. Within the PV, 5-HTR2A had relatively more mRNA than 5-HTR1B ($P = 0.01$). In the UA, 5-HTR2B had the greatest relative quantity of mRNA ($P < 0.0001$), while 5-HT2A had a lower relative quantity of mRNA than 5-HT2B ($P < 0.0001$) or 5-HT1D ($P = 0.0003$). Relative differences in vessel receptor types emphasize the different physiological roles of the vessels under normal physiological conditions. Additionally, different receptor types may also contribute to the different capacities of the vessels to respond to, or be protected from, vasoconstrictive effects of fescue toxicosis in horses.

Key Words: equine, fescue, vasoconstriction

TH272 Use of kainic acid as an alternative to commercially available anthelmintics in horses. K. J. Stutts^{*}, J. L. Lucia, M. J. Anderson, S. D. Brooks, and M. L. McMillan, Sam Houston State University, Huntsville, TX.

Parasite management is important in any equine program. Traditionally, intestinal parasites have been controlled by the routine use of commercial equine anthelmintics. A potential alternative to these products is the use of natural plant extracts containing kainic acid. The objective of this study was to evaluate the efficacy of kainic acid in mitigating intestinal parasite load in young horses. Prior to the study, all horses were treated with fenbendazole at 6 and 12 mo of age and with ivermectin at 18 mo of age. Twelve 2-year-old Quarter horses were blocked by location in barn and randomly assigned to 1 of 2 treatments. Treatments consisted of a one-time oral dosage of 25 mg of fenbendazole per 45 kg BW ($n =$

6), or 60 mg of kainic acid per 45 kg BW (n = 6) provided in a pelleted seaweed extract (Acadian Seaplants, Dartmouth, Nova Scotia, Canada) that was delivered in the ration over a 5-d period. All horses were housed in 3 × 3 m stalls, were exercised daily, and received a commercially available 14% pelleted concentrate and approximately 1% BW/d of Coastal bermudagrass (*Cynodon dactylon*) hay at 12-h intervals. Fecal samples were obtained via rectal palpation at d 0 and d 7, 14, 21, 28, and 35 following treatment. All samples were stored at 4°C before fecal egg analysis via the Modified Wisconsin Sugar Flotation procedure. An ANOVA was conducted to determine differences in mean fecal egg counts (FEC) between treatments. No differences were detected in FEC between treatments except at d 35 when the mean FEC for the fenbendazole group (22.50) was lower ($P < 0.03$) than that of the kainic acid group (80.17). Maximum FEC reduction of 85.08% was observed in the fenbendazole group at d 14 post treatment, and maximum FEC reduction of 87.94% was observed in the kainic acid group at d 7 post treatment. These results indicate that the seaweed product containing kainic acid was as effective as fenbendazole in reducing FEC in 2-year-old Quarter horses throughout the majority of the 35-d trial. However, efficacy of fenbendazole may be sustained longer as mean FEC of the kainic acid group was greater on d 35 after treatment.

Key Words: horse, anthelmintic, kainic acid

TH273 Comparison of on-farm and commercial laboratory fecal egg counts to determine internal parasite status of horses.

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Techniques implementing free-swing centrifugation for fecal egg analysis have been documented as superior methodology for accurate

determination of parasite species and egg count. However, determining the efficacy of on-farm methodology is limited. The objectives of this study were to determine the effectiveness of 2 commercially-available anthelmintics, and to determine the efficacy of on-farm evaluation of internal parasite status in horses. Sixteen 2-year-old Quarter horses were blocked by location in barn and randomly assigned to one of 2 treatments. Treatments consisted of a one-time oral dosage of 25 mg of fenbendazole per 45 kg BW (n = 8) or 200 µg ivermectin per kg BW (n = 8). All horses were housed in 3 × 3 m stalls and received a commercially available 14% pelleted concentrate and approximately 1% BW/d of Coastal bermudagrass (*Cynodon dactylon*) hay at 12-h intervals. Fecal samples were obtained via rectal palpation at d 0 and d 7, 14, 21, and 28 following anthelmintic dosing. All samples were stored at 4°C before fecal egg analysis. Samples were sent to a commercial laboratory (MidAmerica Agricultural Research Center, Verona, Wisconsin) and analyzed on-farm for fecal egg counts (FEC) utilizing the Modified Wisconsin Sugar Flotation procedure. An ANOVA was conducted to determine differences in mean fecal egg counts (FEC) between treatments and laboratories. Mean FEC were not different ($P > 0.11$) between anthelmintic treatments at the initiation of the study, however, ivermectin decreased ($P < 0.01$) FEC beginning at d 7 when compared with fenbendazole administration. In addition, mean FEC did not differ ($P > 0.05$) between the commercial and on-farm laboratories. These results indicate that ivermectin was more effective in reducing intestinal parasite load in 2-year-old Quarter horses than fenbendazole. Additionally, the Modified Wisconsin Sugar Flotation procedure is effective in determining internal parasite status of horses on the farm and requires minimal experience by the individual utilizing the technique.

Key Words: horses, anthelmintics, Modified Wisconsin Sugar Flotation