HORSE SPECIES I

1198 (T136) Glucose-insulin homeostasis and characterization of proteins involved in glucose uptake signaling in equine skeletal muscle. R. C. Avenatti*, K. Malinowski, and K. H. McKeever, *Rutgers Equine Science Center, New Brunswick, NJ.*

The objective of this study was to test the hypothesis that glucose-insulin homeostasis, and activation of AMP-activated protein kinase (AMPK), the protein kinase Akt, and the Akt substrate protein of 160 kDa (AS160) in equine skeletal muscle are altered by acute, exhaustive exercise and by aging. Unconditioned aged (n = 6; 22.6 ± 2.25 yr) and young (n = 6; 5.5 ± 2.8 yr) Standardbred mares were assessed for glucose-insulin homeostasis via frequently sampled intravenous glucose tolerance test (FSIGTT). All mares underwent a single bout of submaximal exercise. Plasma insulin and glucose concentrations were measured via radioimmunoassay and enzyme-electrode interface, respectively. Mid-gluteal muscle biopsies were taken pre-exercise, and at 0, 4, 24, and 48 h post-exercise. Muscle samples were analyzed via western immunoblotting for changes in activation of AMPK, Akt and AS160. Minimal model analysis of FSIGTT and repeated measures ANOVA were utilized to analyze data. Null hypothesis was rejected when $P < 0.05$. FSIGTT results indicated that there was no difference between young and aged mares for insulin sensitivity (SI), glucose effectiveness (SG), acute insulin response to glucose (AIRg) or disposition index (AIRg x SI) ($P > 0.05$). Area under the curve for both insulin (AUCi) and glucose (AUCg) were not different between young and aged mares ($P > 0.05$). In response to acute exercise, young mares displayed elevated insulin concentrations at 2 ($P = 0.009$) and 4 ($P = 0.007$) h while aged mares displayed elevated insulin at 30 ($P < 0.001$) and 60 ($P = 0.001$) minutes post-exercise. Neither age nor exercise caused a significant change in AUCi ($P > 0.05$). Glucose concentration was elevated at 2 h post-exercise in young mares ($P < 0.001$), while in aged mares glucose remained elevated only until 60 min post-exercise ($P = 0.037$). Exercise caused an increase in AUCg in young ($P = 0.007$) and aged ($P = 0.031$) mares, however there was no age effect on AUCg ($P > 0.05$). Neither age nor exercise altered total protein concentrations or phosphorylated protein concentrations of AMPK, Akt or AS160 ($P > 0.05$). In conclusion, age alone is not sufficient to alter insulin sensitivity in horses, but does alter glucose-insulin dynamics in response to exercise. Also, a single bout of submaximal exercise was not sufficient to alter activation of proteins believed to be involved in glucose uptake in skeletal muscle at the time points measured. The comparative literature suggests that these proteins are important for endocrine- and exercise-related glucose uptake and energy homeostasis.

Key Words: aging, exercise, muscle

1199 (T137) Splanchnic extraction of phenylalanine in adult thoroughbred mares fed two different levels of threonine. S. Tanner, T. Barnes, K. Cybulak, and K. L. Urschel*, *University of Kentucky, Lexington.*

Previous studies in horses examining the effects of amino acid (AA) intake on whole-body protein synthesis have used intravenous phenylalanine isotope infusion and the resulting calculations required estimates of splanchnic phenylalanine extraction that were derived from other species. Threonine is believed to be the second limiting AA in some equine diets. The objectives of the study were to determine splanchnic extraction of phenylalanine, validate an oral infusion of [1-^{13}C]phenylalanine, and test the effects of threonine supplementation on whole-body protein synthesis in horses. Six thoroughbred mares were fed timothy hay and a low threonine concentrate supplemented with isonitrogenous amounts of either threonine (+THR) or glutamate (+GLU), which were top dressed on the concentrate portion of the diet. Threonine intakes were 119 (+THR) and 58 mg/kg/d (+GLU) and diets exceeded NRC recommendations for all nutrients, including threonine (33 mg/kg/d). Each horse received each diet twice for 7d; studied once with an oral infusion and once with an intravenous infusion of [1-^{13}C]phenylalanine, for a total of four study periods per horse. On d 6 of receiving each diet, blood samples were taken before and 90 min after the AA supplemented concentrate meal. The next day, a 2-h primed, constant intravenous infusion of [^{13}C]sodium bicarbonate and a 4-h primed, constant infusion of [1-^{13}C]phenylalanine, either oral or intravenous, were used with blood and breath sampling to measure blood [^{13}C]phenylalanine and breath $^{13}CO_2$ enrichment. Data were analyzed in the PROC MIXED of SAS with threonine intake, route of infusion and the interaction as the fixed effects. Baseline and post-feeding plasma concentrations of glutamate, serine, glycine, threonine, and methionine were affected by diet ($P < 0.05$). Phenylalanine flux, intake, from protein breakdown, oxidation, and non-oxidative disposal were not affected by diet ($P < 0.05$). Splanchnic extraction was 26 ± 5% and 27 ± 3% for the +THR and +GLU diets, respectively. This was the first study to use oral administration of [1-^{13}C]phenylalanine in horses and this technique offers a less invasive alternative to the intravenous infusion method. Threonine does not appear to be a limiting AA in our diet, as phenylalanine kinetics were not affected by supplementation; however differences in plasma AA concentrations in response to threonine supplementation suggest that this AA can affect the metabolism of other AA. *This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2012–67015–19448 from the USDA National Institute of Food and Agriculture.*

Key Words: splanchnic extraction, equine, amino acids

1200 (T138) Effects of a docosahexaenoic acid-rich algae supplement on plasma amino acid levels in healthy, mature horses after prolonged treatment with dexamethasone. R. A. Williams[1], K. L. Urschel[2], R. E. Schaeffer[1], and K. M. Brennan*[1], *[1]Alltech Inc., Nicholasville, KY, [2]University of Kentucky, Lexington.*

Dexamethasone (DEX) is used to treat inflammation and off-label as a calming agent in performance horses. Long-term treatment with DEX reduces insulin sensitivity (SI) and can impair other insulin-activated pathways such as protein synthesis. We previously reported that supplementation with docosahexaenoic acid (DHA)-rich algae increased SI during prolonged DEX treatment in healthy horses. The objective of this study was to determine the effect of a DHA-rich algae supplement on plasma amino acid levels in healthy, mature horses after prolonged treatment with DEX. Eight healthy, mature horses were used in a balanced, crossover design of two 56-d periods. In each period, horses were fed a basal diet (CON), or diet + 152 g/d of a DHA-rich algae (15% CP; Algae SP-1, Alltech Inc., (ALG)) providing 21.1 g/d DHA for 28 d, after which blood was sampled (*P*re-DEX) and 0.04 mg DEX/kg BW/d administered orally; treatment continued for 21 d, then blood was sampled (*P*ost-DEX). Plasma AA concentrations were determined every 30 min during a 2-h insulin infusion. Plasma amino acid concentrations were analyzed using PROC MIXED using repeated measures analysis, with pre-DEX baseline as a covariate. Diet had no effect on plasma amino acid concentrations with the exceptions of Lys, Met, Ala and Gln ($P < 0.05$). A treatment by DEX interaction ($P < 0.01$) occurred for Glu, Ser, Asn, Gly, Gln, His, Thr, Ala, Pro, Tyr, Val, Met, Leu, Phe and Lys. Within each of the CON and ALG treatments, all horses received the same diet during both the pre- and post-DEX periods and therefore the increase in plasma amino acid concentrations in response to DEX in the CON horses suggests either an increase in rates of protein degradation or a decrease in rates of protein synthesis in response to prolonged DEX administration. Daily ALG feeding appeared to mitigate these changes in plasma amino acid concentrations. Additional research is necessary to identify the mechanism of this algae effect, although we hypothesize this is due to the previously reported increased SI with ALG.

Key Words: horse, amino acid, dexamethasone, docosahexaenoic acid

1201 (T139) Evaluating the expression of microRNA miR-1 and miR-133 in the muscle of horses fed a docosahexaenoic acid-rich algae supplement after prolonged dexamethasone treatment. M. L. Spry, A. C. Smith, D. E. Graugnard, R. E. Schaeffer, and K. M. Brennan*, *Alltech Inc., Nicholasville, KY.*

MicroRNAs (miRNAs) are small noncoding RNAs that bind to the 3' untranslated (3'UTR) region of genes and are responsible for inhibiting translation or promoting mRNA degradation. Two well characterized muscle-specific miRNAs are miR-1, and miR-133. MiR-1 decreases myoblast proliferation and increases differentiation, whereas miR-133 has an opposing effect promoting myoblast proliferation while decreasing differentiation. It is known that skeletal muscle breakdown and an increase in circulating amino acids are associated with the decreased insulin sensitivity after prolonged dexamethasone (DEX) treatment. In horses there is an association between an increase in miR-1 and miR-133 levels and diseases involving muscle breakdown. Supplementing diets with DHA-rich algae (Algae SP-1, Alltech Inc., (ALG)) improves insulin sensitivity and decreases plasma amino acid levels in horses after prolonged DEX treatment. The objective of this study was to determine the effect of Algae SP-1 supplementation on miR-1 and miR-133 expression in healthy, mature horses ($n = 5$) before and after DEX treatment using a balanced, crossover design of two 56-d periods. In each period, horses were fed a basal diet (CON), or diet + 152 g/d of ALG for 28 d and muscle was sampled (*P*re-DEX). DEX was then administered (0.04 mg/kg BW/d, orally) for 21 d while dietary treatments continued. On d 49 of dietary treatments (*P*ost-DEX), muscle was sampled and microRNA was measured using TaqMan MicroRNA Assays (Applied Biosystems). The relative quantification (RQ) was expressed as a ratio of the target microRNA to control microRNA normalized to a pooled control (CON, pre-DEX) using the delta-delta Ct ($^{\Delta\Delta}$Ct) method. There was no effect of diet, DEX or diet*DEX interaction on skeletal muscle miR-1 and miR-133 expression. Novel techniques used for miRNA evaluation could be useful to help relate changes in gene expression to physiological status, however further studies are needed.

Key Words: microRNA, horse, dexamethasone, docosahexaenoic acid

1202 (T140) The effects of abrupt dietary alterations on equine cecal pH. A. Reeg*[1], T. Douthit[1], K. M. DeLano[1], M. E. Gordon[2], M. M. Raghavendra Rao[2], and K. Williamson[2], *[1]Kansas State University, Manhattan, [2]Purina Animal Nutrition, LLC, Gray Summit, MO.*

Feeding starch in quantities exceeding 3.4 g/kg BW to the equine has been shown to exceed small intestinal capacity for digestion (Potter et al., 1992). This leads to fermentation of starch in the cecum resulting in a buildup of acidic products and decline in pH. Two consecutive 22-d experiments separated by 2 d of rest were conducted to identify whether abruptly feeding 1% (3.1 g starch/kg BW) and 1.25% (3.88 g starch/kg BW) BW concentrate during experiments 1 and 2, respectively, without hay would elicit a more profound decrease in cecal pH, as compared to the baseline diet. Nine cecally cannulated 8- to 10-yr-old quarter horses, 5 geldings and 4 mares, ranging in BW from 455 to 591 kg, were utilized. Baseline diets

for both expt. consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO; 1.55 g starch/kg BW) fed at 0700 and 1.5% BW native prairie grass hay divided into two feedings (0700 and 1930) for 21 d. On d 22 of both experiments the concentrate meal was increased to 1% (Exp. 1) and 1.25% (expt. 2) BW and fed without hay. Cecal pH was measured from d 19 to 22 of both experiments at -1, +1, +4, +8, +12, +16, +20, and +24 h relative to feeding concentrate. ANOVA was performed with mixed models (SAS 9.3, 2011) and least square means compared using Fisher's LSD ($P < 0.05$, LSM ± SE). Complete randomization with either repeated measures (Exp. 1) or a split-plot design (expt. 2) was utilized. In both experiments, there was a time effect ($P < 0.0001$) on cecal pH. Exp. 1 cecal pH at +1 (7.2 ± 0.046) and +12 (6.8 ± 0.046) h on d 22 was decreased ($P < 0.05$) when compared to mean responses at +1 and +12 h during baseline feeding (7.4 ± 0.046 and 7.02 ± 0.046, respectively). In Exp. 2 cecal pH was decreased ($P < 0.05$) only at +12 h (6.8 ± 0.041) on d 22 when compared to mean values at +12 h during baseline feeding (7.0 ± 0.041). Throughout both experiments, post-prandial cecal pH was characterized by a decline at +4 h, reached a minimum value at +8 h, and increased by +12 h. However, the primary variation between baseline and d 22 was a more rapid rise in pH from +8 to +12 h.

Key Words: equine, starch, cecal pH

1203 (T141) Utilizing fecal pH to predict cecal pH in the equine. C. J. Douthit*[1], T. Douthit[1], A. Reeg[1], N. M. Bello[1], M. E. Gordon[2], and K. Williamson[2], *[1]Kansas State University, Manhattan, [2]Purina Animal Nutrition, LLC, Gray Summit, MO.*

Cecal cannulation is commonly used for direct assessment of dietary manipulation on cecal pH in equines. However, analysis of fecal material is considered one of few viable methods for monitoring digestive health in intact horses. In this retrospective study, we assessed the association between fecal pH and cecal pH in an attempt to develop a predictive equation for cecal pH. Nine cecally cannulated quarter horses were utilized. The group was comprised of 5 geldings and 4 mares, between the ages of 8 to 10 yr and body weight ranging from 455 and 590 kg. Horses were housed in heated individual stalls with ad libitum access to water and white salt blocks. Horses were fed 1.5% BW prairie grass hay split into twice daily feedings (0700 and 1830) and 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) which was fed in the mornings only (0700). Horses were maintained on this diet for 3 separate 21-d periods. Cecal and fecal pH were measured on d 19 to 21 of each period at -1, +1, +4, +8, +12, +16, +20, and +24 h relative to feeding the concentrate meal. Given inherent animal-to-animal variability in the pH dynamics of the cecum and rectum over time, the minimum cecal pH after feeding was collected for each animal-day combination and modeled as a function of the corresponding minimum fecal pH using a general linear mixed model. Analysis revealed evidence for an association ($P = 0.03$) between minimal cecal pH and minimal fecal pH. The estimated rate of change for minimum cecal pH per unit increase in minimum fecal pH was 0.131 with 95% confidence interval [0.011, 0.251]. The prediction equation for estimated cecal pH was $Y = 0.131*X + 5.8969$, where X is the observed fecal pH. However, the amount of predictive variability was considerable, likely due to multiple factors contributing to cecal pH, including feed composition, animal weight, and physiological state, which can alter the transit time of digesta from cecum to rectum. Our data indicates that fecal pH appears to have limited usefulness in predicting cecal pH.

Key Words: cecal pH, equine, fecal pH

1204 (T142) Comparison of ultrasound transducers to determine rump fat thickness in mature horses at maintenance. K. J. Stutts*, J. L. Lucia, M. J. Anderson, M. M. Beverly, and S. F. Kelley, *Sam Houston State University, Huntsville, TX.*

Rump fat measurements are utilized to assess changes in subcutaneous fat thickness when conducting feeding trials in horses. Commonly, these measurements are taken using a transducer that is designed for use in the rectum to evaluate the reproductive tract. This study was conducted to determine if measurements obtained using a rectal transducer were comparable to measurements obtained using a transducer designed to measure carcass characteristics. Rump fat measurements were obtained from 30 mature horses (368 to 552 kg and 5 to 10 yr) that were part of a feeding trial. Measurements were taken at 28-d intervals for a period of 154 d at a point half way between the points of the hip (Tuber coxae and Tuber ischiadicum) and 6 cm from the midline of the horse with the transducer positioned perpendicular to the midline of the horse. Images were taken with an Aloka SSD 500V real-time ultrasound machine (Corometrics Medical Systems, Wallingford, CT) equipped with a 17.2-cm, 3.5-MHz linear transducer (carcass probe) and a 6-cm, 5-MHz linear transducer (rectal probe). All measurements using the carcass probe were conducted by a certified technician who captured images that were analyzed at the Centralized Ultrasound Processing Lab (Ames). All measurements using the rectal probe were conducted by a non-certified employee using on-screen diagnostic tools on the scanner. Longissimus muscle (LM) area between the 17th and 18th ribs and fat thickness three-quarters the length ventrally over the LM were also obtained using the carcass probe. Least squares means for rump fat thickness (RFT) and LM fat thickness (LMFT) were calculated using the GLM procedure of SAS. Pearson correlation coefficients were used to determine the relationship between RFT and LMFT. Mean RFT was greater ($P < 0.01$) using the rectal probe (1.27 ± 0.39 cm) compared to the carcass probe (1.00 ± 0.36 cm); however, there was a strong, positive relationship between the two measurements

(r = 0.76, P < 0.01). Measurements using both the rectal (r = 0.48) and the carcass (r = 0.33) probes also had a positive relationship (P < 0.01) with LMFT with a greater correlation observed between LMFT and RFT measurements using the rectal probe. These data validate the utility of the rectal probe to measure RFT in horses. Although measurements were different between the rectal and carcass probes, measurements obtained using the rectal probe accurately assessed changes in RFT and exhibited a greater correlation to changes in fat thickness over the *longissimus* muscle.

Key Words: horses, ultrasound, rump fat

1205 (T143) On-farm tapeworm testing in horses.

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The incidence of tapeworm infection in horses was investigated on nine farms in North Carolina and one in Virginia. Two of the North Carolina farms were used in three replicates. A total of 602 horses at least 1 yr of age were used (321 individuals, 281 were replicates; 157 females, 164 males) in the summer, fall, and winter seasons. Individuals averaged 12.1 ± 0.4 yr of age and 541.8 ± 7.0 kg body weight. Animals were treated with commercial horse anthelmintics containing ivermectin and praziquantel (Zimecterin Gold; n = 412; eight farms including those replicated; and Equimax™; n = 190; four farms) as labeled for BW measured by equine weight tape plus 15% with doses rounded up to the nearest 22.7 kg (50 lb). At treatment (d 0) and 24 hlater (d 1), fecal samples were collected after defecation for tapeworm fecal egg count (FEC) using the Modified McMasters technique with a sensitivity of eight eggs per gram (epg). Descriptive statistics were used to report data. On d 0, 1.4 ± 0.01% of horses were positive for tapeworm eggs, whereas on d 1, 20.6 ± 1.7% were positive. Positive horses were found on 30% of farms on d 0 and 60% of farms on d 1. This data suggests that tapeworm infections are common on horse farms, but that most horses do not have infections or have undetectable infections. Furthermore, these data demonstrate that sensitivity of detection for tapeworms via fecal exam is greatly increased by testing 1 d after treatment.

Key Words: tapeworms, horses, FEC