301 Effect of ryanodine, nifedipine, and low sodium on contracture force in isolated muscle bundles from horses with recurrent exertional rhabdomyolysis. G.A. Searls* and G.W. Onan, *University of Wisconsin River Falls, Wisconsin*.

Previous studies have shown that isolated muscle bundles from Thoroughbred horses with an inherited form of Recurrent Exertional Rhabdomyolysis (RER) develop contractures in the presence of either halothane or lower concentrations of caffeine than isolated bundles from normal horses. Therefore contracture tests similar to those used for identification of humans subject to malignant hyperthermia have been developed for early identification of foals carrying the RER gene. The purpose of this study was to determine if any other substances might have a differential effect on *in vitro* contractures of muscle bundles from RER vs. normal horses in order to better refine diagnoses of foals and to further indicate a potential source for the defect in RER muscle. A series of pharmaceuticals known to have effects on calcium channel proteins or calcium transport proteins were investigated. Muscle bundles from RER horses developed significantly stronger contractures in the presence of 0.05 μM and 0.1 μM ryanodine (P<.05) than did bundles from normal horses. This is consistent with the reaction of malignant hyperthermia muscle from human and swine subjects, offering further evidence that the RER condition in Thoroughbreds is a related disease and that the defect lies in the calcium buffering ability of the sarcoplasmic reticulum. A further series of studies investigated the effects nifedipine (a dihydropyridine calcium channel blocker) and low extracellular sodium (which affects the sarcolemmal sodium-calcium transporter protein) on the extent of caffeine-induced contractures in RER vs. normal muscle bundles. The presence of 10 μM nifedipine or low (15mM) sodium both caused increased contracture force at 1.0 mM, 2.0 mM, and 5.0 mM caffeine (P<.05) in muscle bundles from all horses with no differential effect between types. These results would indicate that sarcolemmal calcium regulating proteins are not important in the etiology of RER onset in affected horses and further substantiate that the defect lies with the sarcoplasmic reticulum. Furthermore ryanodine may be a useful adjunct in early diagnosis of the disease in foals.

Key Words: Recurrent Exertional Rhabdomyolysis, Ryanodine, Nifedipine

303 A comparison of different particle size analysis techniques. A.L. Baldridge*, T.L. Stainbrook, J.C. Woodworth, M.D. Tokach, J.L. Nelssen, R.D. Goodband, and S.S. Dritz, *Kansas State University, Manhattan*.

Particle size of ground grain is determined in labs using the ASAE approved, 13-sieve method. Because this method is time consuming, a 1-sieve, hand-shaking method has been developed (1s; IFA, Stanly, IA). Questions on the accuracy of the 1-sieve method led us to develop an alternative method to quickly determine particle size with 3 sieves and to compare the various methods. Forty-three samples of ground corn were analyzed by the approved 13-sieve procedure (13s; 3350 μm to 53 μm opening mesh screens) with mean particle size of samples ranging from 1143 to 422 μm. The same samples were analyzed by the 1s method and the developed 3-sieve (3s) method. For 13s, the approved ASAE protocol was followed: 100 g of corn was placed on top of the sieve stack and shaken for 10 min on a Rotap sieve shaker. For 1s, IFA protocol was followed: 280 g of corn was placed on a sieve (1400 μm opening) and shaken by hand until no more sample fell through. For 3s, 50 g of sample was placed on top of a stack (1700, 600, and 300 μm opening) and shaken by hand for 1.5 min. Mean particle size was calculated based on the amount of sample resting on each screen after shaking. For 1s, the IFA procedure also was compared to a new prediction equation (11.86×wt on screen, g + 435; R=0.74). For 3s, the prediction equation was 18.89×(X1700) + 10.87×(X600) + 1.18×(X300) # 150 (R=0.88) where X equals the percentage of sample on the respective screens. The different methods were compared by calculating the residual of the predicted particle size from the particle size determined from the 13s method. The residual for 3s (44 πm) was lower (P < 0.01) than 1s using both the IFA protocol (133 μm) and the new prediction equation (74 μm). Residual of 1s using the prediction equation also was lower (P < 0.01) than 1s using IFA protocol. In conclusion, 3s is a quick method that can be used to predict particle size with less variation than the 1s method commonly used. The 1s method can be improved by using a different prediction equation than that provided by the company.

Key Words: Grain, Particle size, Procedures

304 Effect of ruminal protein degradability and supplementation frequency on intake, diet digestibility, and nitrogen balance in forage-fed lambs. D. E. Carter*, P. A. Ludden, V. Nayigihugu, and B. W. Hess, *University of Wyoming*.

Twenty-four wether lambs (initial BW = 36.8 ± 0.7 kg) were used in a 56 d split block, 2 × 2 factorial designed experiment to evaluate the effects of ruminal protein degradability (RDP) and supplementation frequency on intake, diet digestibility, and N retention. All lambs were fed chopped (7.6 cm) bromegrass hay (7.4% CP, 61.1% RDP, 59.3% NDF, 33.7% ADF) for ad libitum consumption, and either soybean meal (high RDP) or feather meal (low RDP) daily or on alternate days. Supplements were fed on an isonitrogenous basis (0.28 and 0.20% of BW daily for the high and low RDP supplements, respectively), with alternate-day supplements fed at twice the level of daily supplementation. Beginning on d 21 and 49 of the trial, two 8-d N balance collections were conducted. No protein degradability × supplementation frequency interactions ($P \geq 0.24$) were noted in this experiment. No treatment effect was noted for forage DM intake ($P \geq 0.21$), total DM intake ($P \geq 0.08$), N intake ($P \geq 0.79$), or total tract DM digestibility ($P \geq 0.10$). Total tract N digestibility was not affected ($P \geq 0.42$) by protein degradability, but was increased ($P = 0.01$) with alternate day supplementation (57.2 vs 54.6%). A protein degradability × collection period interaction was observed for N retention (g/d and % of N intake; $P \leq 0.05$), wherein feeding the low RDP supplement produced a greater increase in N retention during the second collection period. Overall, protein degradability did not affect ($P \geq 0.29$) urinary N excretion or N retention; however, alternate day supplementation decreased ($P = 0.02$) urinary N excretion, thereby increasing N retention in g/d (6.57 vs 5.32; $P = 0.01$), as a % of N intake (33.8 vs 27.0; $P = 0.003$) and as a % of digested N (58.8 vs 49.4; $P = 0.01$). Supplementing protein to forage-fed ruminants on alternate days appears to enhance efficiency of N utilization, irrespective of ruminal protein degradability.

Key Words: Ruminal Protein Degradability, Supplementation Frequency, Nitrogen Retention

305 Effects of harvest date and late-summer fertilization rate on dry matter yield and chemical composition of stockpiled bermudagrass forage. A.A. Gelvin*[1], D.L. Lalman[1], C.F. Taliaferro[1], and J. Ball[2], [1]*Oklahoma Agricultural Experiment Station,* [2]*Noble Foundation, Ardmore, OK*.

A randomized complete block design with four replications was used to test the effects of N fertilization rate and harvest date on yield and chemical composition of stockpiled Greenfield bermudagrass at the Eastern Research Station near Haskell, OK. Four N fertilization rates were applied on August 17, 1998 (0, 34, 67, 135 kg/ha) and forage was sampled at five 28-d intervals beginning on November 5. During late April, prior to the experiment, 112 kg N/ha was applied and P & K was applied as indicated by soil test. Hay was harvested from the plots during early June and again during early August. Near infrared reflectance spectroscopy (NIRS) was used to determine chemical composition including CP, soluble protein (SP), neutral detergent insoluble crude protein (NDICP), ADF, NDF, lignin (LIG), non-structural carbohydrate (NSC), fat, and ash. Total digestible nutrients was calculated using the summative approach. Fifteen percent of the samples were analyzed for each component using wet chemistry procedures to calibrate the NIRS equations. Degradable protein concentration (DIP) was determined using the *Streptomyces griseus* enzymatic procedure. Monthly precipitation was 16.1, 21.4, 9.2, 5.9, 6.6, and 5.8 cm for September, October, November, December, January, and February, respectively. Forage dry matter yield, determined on November 5, increased linearly (P < .01; y = 37.454x + 3120) with increasing N fertilization. Concentration of CP, SP, DIP, NDICP, and TDN increased (P < .05) with increasing N fertilization. However, concentration of cell wall constituents and fat decreased (P < .01) with increasing N fertilization. Ash and NSC were not affected by N fertilization rate (P > .2). As the winter progressed, concentration of CP, SP and NDICP decreased (P < .01), although DIP increased (P < .01) over time. Later harvest dates were associated with increased (P < .01) ADF and NDF concentration and decreased (P = .01) NSC, with no change in LIG or TDN (P > .2). Increased N fertilization resulted in greater stockpiled bermudagrass yield and nutritive value, although the effects of harvest date were variable.

Key Words: Stockpiled Bermudagrass, Forage Nutritive Value, Protein Fractions