muscle fiber types. We have found that in adult chicken pectoralis muscle, the tapered ends of fibers contain the neonatal myosin heavy chain isoform in addition to the adult isoform found throughout the length of the fiber. We have made similar observations studying mature pigeon muscle. Examination of serial sections along the length of muscle fibers of chicken pectoralis at different stages of development, illustrated that the transition from the neonatal to adult myosin heavy chain isoform proceeds as a gradient from the centrally located motor endplate towards the ends of a muscle fiber. Additional evidence demonstrates differences in the amount of cytoplasm per nucleus at the ends of chicken pectoralis muscle fibers throughout development. In denervated adult fibers, those myonuclei furthest from the endplate are the first to re-express neonatal myosin. We examine the hypothesis that during development neonatal myosin is initially suppressed near the motor endplate and that trophic factors emanating from the vicinity of the motor endplate represent a potential localized signaling pathway that may differentially modulate myosin heavy chain gene expression in nuclear domains along the length of the muscle fiber.

**Key Words:** Skeletal Muscle, Myosin, Heterogeneity

### 679 Calcineurin and NFAT signaling in myogenesis.

G.K. Pavlath*, *Emory University, Atlanta, GA USA*.

Adequate muscle mass is crucial for the maintenance of functional muscle tissue. However, the signaling pathways that control muscle growth are unclear. The nuclear factor of activated T cells (NFAT) proteins are a family of transcription factors whose activation is controlled by calcineurin, a Ca2+ dependent phosphatase. Calcineurin and NFAT have distinct functions in multiple steps of myogenesis. Several NFAT isoforms are expressed in skeletal muscle. However, individual NFAT isoforms are activated during different stages of muscle development. Specifically, the NFATC2 isoform is activated only in newly formed myotubes. We have shown that adult NFATC2-/- mice exhibit reduced muscle size due to a decrease in the cross sectional area of individual myofibers, suggesting that muscle growth is blunted. The growth defect is intrinsic to muscle cells as the lack of NFATC2 in primary muscle cultures results in reduced cell size and myonuclear number in myotubes. These results implicate a novel role for the transcription factor NFATC2 in regulating skeletal muscle growth. Additional evidence suggests that NFATC2 either directly or indirectly regulates the expression of a secreted molecule that regulates muscle growth. Media conditioned by wild-type myotubes contains bioactivity that can rescue the reduced cell and myonuclear number defect of NFATC2-/- muscle cells. IGF-1, a known regulator of muscle growth, does not mimic the effects of conditioned media. Biochemical characterization and fractionation of conditioned media from wild-type myotubes suggests that the factor is heat and protease sensitive with a molecular weight around 10kD. These studies may lead to the identification of novel factors that regulate muscle growth. Such factors may be useful for inducing muscle growth during periods of muscle loss associated with disease, disuse, or aging.

**Key Words:** muscle growth, growth factors, calcineurin

### 680 Calcineurin signaling in skeletal muscle growth.

R.N. Michel*, *Laurentian University, Sudbury, Ontario, Canada*.

The adult motor nerve confers to its target skeletal muscle fibers distinct morphological, biochemical and functional characteristics. This is not only achieved via nerve-mediated electrical activation signals but also by means of nerve- and muscle-derived trophic mechanisms. Though our understanding of these neural influences is rudimentary there is mounting evidence that the calcium/calmodulin-dependent phosphatase calcineurin is an important molecular signaling intermediate in this modulation. Specifically, calcineurin appears as a crucial link between upstream activity-related effectors and its specific downstream targets involved in the regulation of the muscle cell phenotype, and in particular, cellular size. Though the positioning of calcineurin at the nexus of growth is currently under debate (S. Dunn, A. Simard, R. Prudhomme and R.N. Michel, Nature Cell Biology 4: E46-47, 2002), its role in modeling skeletal muscle phenotype is uncontested. In this presentation, I will: 1) define the role of calmodulin-calcineurin signaling in the modulation of size and phenotype of all (fast and slow) skeletal muscle fiber types and in the promotion of satellite cell differentiation in vivo, 2) provide insight into the contribution of neural-linked upstream signaling and contractile loading factors in the activation of the calcineurin pathway, specifically our model of 'frequent muscle usage' above 'native' muscle activity levels as a prerequisite for calcineurin signaling, 3) provide evidence for the contribution of parallel signaling pathways cooperating with calcineurin during growth, and 4) identify the cellular substrates and gene targets of this phosphatase. Supported by NSERC Canada.

**Key Words:** nerve activity, cell signaling, muscle phenotype

## Horse Species
## Horses: Livestock Versus Companion Animals - Implication for Animal Agriculture

### 681 Horses ARE companions, but...

M.A. Russell*, C.M. Brady, E.A. Pajor, and A.M. Beck, *Purdue University, West Lafayette, IN USA*.

Internationally, horses have a strong affiliation with agrarian life and serve humans as food, war machines, transportation, and beasts of burden. They are included in Webster's definition of livestock: "domestic animals, such as cattle, horses, sheep, hogs, or goats, raised for home use or for profit". Many farmers, cowboys, and cavalry soldiers consider their horses as their co-workers and faithful companions. In North America, only 10-20% of the horses are owned with a commercial industry or profit motive (AHC, 1999) with availability of discretionary income determining the demand for horses. This is a function of the general, not the agricultural economy, and input suppliers and veterinarians will confirm that the majority of horseowners approach expenditures with a mentality more similar to cat or dog owners than to production farmers. Beck and Katcher (1996) reported that 87% of US Pony Club members surveyed responded that horses were part of the family and Melson (2001) reported that children "liked their horse better than a good friend". Webster defines pet as "any object kept for the affection, an animal kept for amusement or companionship, something cherished or a favorite". In interviews with youth ages ten to fourteen, 75% indicated that when they were upset they turned first to their horses (Covert et al., 1985). So are horses companion animals? They are big business! There is no doubt about the companion relationship between most horseowners and their horses, but politically we want to maintain the connection and importance of the horse industry to the agricultural business sector and heritage. There are justifiable legal, political, and economic reasons for this position, however, the future viability of departments of animal sciences and veterinary medicine depends on how they respond to the changing demographics of our society. We must relate to an increasing number of people in a companion relationship with their animals in order to attract youth to our scientific disciplines, whether we wish to call them livestock or not.

**Key Words:** Companion animals, Horses

### 682 The California perspective- politics, reality, and society.

C. Stull*, *Univsersity of California, Davis, CA*.

Of the estimated one million horses residing in California, more than 3,000 were shipped out of state in 1997 for slaughter and sales as horsemeat for human consumption. Public opinion polls showed that California voters overwhelmingly oppose horse slaughter and oppose eating horse meat. A grass-roots organization, "Save the Horses," was founded to expose the "horrors" of horse slaughter. The Save the Horses proponents developed Proposition 6 for the November 1988 California ballot, which was successfully passed by 60 percent of the voters. Proposition 6 was entitled "Prohibition of Horse Slaughter and Sale of Horsemeat for Human Consumption Act of 1998," and made it a felony to possess, transfer, receive or hold any horse, pony, burro or mule with intent to having it killed for human consumption. Sale of horse meat is prohibited as a misdemeanor offense, with subsequent violations punishable as felonies. Arguments for supporting Proposition 6 contend that historically human beings and horses have enjoyed a special relationship, and believed Californians want to protect their companion and recreational animals from slaughter for human consumption, as has been prohibited legally in the state for cats and dogs. Interestingly, the supporters considered horses as livestock and part of agriculture, but not food animals. The direct impact of Proposition 6 has not been extensively analyzed, but no violations have been recorded. Since 1991, the California Department of Food and Agriculture had the authority to enforce the California Equine Protection Act which included mandatory inspection of all horses leaving the state for slaughter. This program was designed to assist in detecting and recovering stolen horses, and more than 50 horses were recovered through the program in 1997. Since the passing of Proposition 6, this program has been dissolved, thus eliminating this mechanism to recover stolen or missing horses. Other impacts of Proposition 6 are less easily evaluated such as the shipment of horses to slaughter through diverted channels in neighboring states, an increase in the number of abandoned or neglected horses, unacceptable methods of carcass disposal, and the comparatively lower residual value of unwanted or unusable California horses.

**Key Words:** Horses, Livestock, Welfare

**683 Value added equiculture: metamorphosis from livestock to companion.** D. S. Kronfeld*, *Virginia Polytechnic Institute and State University.*

Equiculture has two parts. The primary industry of horse production fits into agriculture. The secondary industry of horse utilization fits into recreation. A value-added benchmark is the 5-fold factor for food in the USA; its value is about 2% of the GNP at the farm gate and about 10% at the human mouth, the added value coming from processing and distribution. The potential for recreation adds much value to horse production. A sound 2-y old Thoroughbred worth $500 of beef-equivalents is commonly worth $2500 for use as a companion animal, 10-times that for sports, 100-times for racing. These factors represent added value at the farm gate, with the economic flux of recreation yet to come. During their agricultural phase, horses are managed as grazing livestock by farmers who regard animals as economic commodities. Care involves improving welfare. During their recreational phase, however, horses are treated as pets by their owners, who often cater to the whims of the animal. Care concerns welfare and the animal's intrinsic rights. These are not the hard rights of extremists but the soft, usually unspoken rights awarded by most people who regard their animals as children and obtain more pleasing performance when recognizing self-awareness and self-interest. This metamorphosis of owner attitude adds value and must be observed by all who serve these owners, such as animal scientists, veterinarians, county wardens, lawyers and legislators. A critical point is reached at the time of disposal, when the horse is no longer serving the best interests of its owner or itself. Is our horse now livestock or pet? Humane societies take dead aim at the disposal of horses as livestock, promulgating horror stories about transport and slaughter. Humane societies also dispose of hundreds of thousands of unwanted dogs and cats in decompression chambers. Would they also decompress horses? More humane methods of euthanasia are approved by the AVMA. Retirement farms postpone the end, but they are expensive and natural death is seldom serene. The equine metamorphosis adds value but hurts at the terminus.

**Key Words:** equiculture, livestock, pet

**684 Evaluation of an introductory course in therapeutic horseback riding at Mississippi State University.** M.C. Nicodemus* and K.M. Holt, *Mississippi State University, Mississippi State, MS/USA.*

With the increased popularity in equine facilitated activities as related to the disabled community, 28 colleges and universities throughout the United States have implemented some form of formal coursework in this new area of equine sciences. Introduction to Therapeutic Riding, ADS 3233, was introduced to the Mississippi State University equine curriculum in the fall of 2001. The three credit hour class included both lecture and riding labs, which aimed to cover various physical and psychological disabilities and different equine activities that addressed the needs of these disabilities. Six students participated in the class (Females: 4; Males: 2) with all pursuing an animal science degree (Bachelors of Science: 5; Masters of Science: 1). All had some form of riding experience and 5 students had volunteered at active therapeutic riding programs. Students answered a 22-question survey on the first (S1) and last (S2) days of class with answers consisting of strongly agree (SA), agree (A), disagree (D), or not applicable (NA). For S1, D was given for 7 of the questions for the majority of the students (67%). Students disagreed with the statements that their confidence were strong in the areas of setting up various therapeutic riding programs, working with the medical and educational community, selecting and training therapeutic riding horses, and finding therapeutic riding resources. Concerning these same questions in S2, 67% of the students either answered SA or A. According to S2, all students indicated a desire to participate in a therapeutic riding program and 33% planned to make a career in this field. 67% of the students suggested more hands-on equine activities for future classes. However, the largest shift in answers from S1 to S2 was in their confidence level concerning the selection and training of therapeutic riding horses (S1: 4 D; S2: 4 SA). From these surveys, development in the course curriculum for this new class can be made to better address the educational needs of the students. This course curriculum will be used as a future guideline for other universities developing a therapeutic riding program.

**Key Words:** Equine Facilitated Activities, Therapeutic Horseback Riding, Undergraduate Equine Curriculum

**685 Dietary grain and endurance exercise.** R. M. Hoffman*[1], T. M. Hess[1], C. A. Williams[1], D. S. Kronfeld[1], K. M. Griewe-Crandell[2], J. E. Waldron[3], P. M. Graham-Thiers[4], L. S. Gay[1], K. E. Saker[1], and P. A. Harris[5], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*High Meadows Farm, The Plains, VA,* [3]*Rectortown Equine Clinic, Rectortown, VA,* [4]*Virginia Intermont College, Bristol, VA,* [5]*WALTHAM Centre for Pet Nutrition, Melton Mowbray, UK.*

Our objective was to observe metabolic and oxidative stress in horses in an 80 km ride, using a 2x2x2 factorial design with grain (<12% versus >17% intake), dietary vitamin E versus vitamins E plus C, and potassium-free electrolyte replacement versus commercial electrolytes with potassium. Methods and results are described in this paper and in two companion papers (Hess et al.; Williams et al.). Blood samples were taken before the race, at veterinary checkpoints 21, 37, 56 and 80 km, and 20 min after finishing. Riders scored horse intakes of food and water at stops, and assigned a performance score. Glucose, electrolytes, partial pressures of oxygen and carbon dioxide, urea, hematocrit, pH, $\alpha$-tocopherol, ascorbate, creatine kinase (CK), aspartate amino transferase (AST), lactate, total protein, albumin, creatinine, cortisol and lipid hydroperoxides (LPO) were analyzed in plasma; glutathione and glutathione peroxidase (GPx) in erythrocytes. Of 46 horses to start, 34 finished, and only their data are used here. During exercise, cortisol, sodium, magnesium, lactate, hematocrit, urea, total protein, creatinine, CK, AST, LPO and GPx increased. Glucose, pH, $\alpha$-tocopherol, chloride and calcium decreased. Hematocrit and lactate were higher ($P = 0.010$) in the fastest horses. Slower horses had lower pH ($P = 0.010$), cortisol ($P = 0.038$), and drank less ($P < 0.0001$) at the finish. Food intake was lower overall ($P = 0.014$) in the fastest horses fed high grain. Performance score was correlated negatively with plasma cortisol, urea, CK, AST, LPO, and positively with glucose.

**Key Words:** Horse, Endurance exercise, Nutrition

**686 Oxidative stress and antioxidant supplementation in horses during a competitive endurance ride.** C.A. Williams*[1], R.M. Hoffman[1], D.S. Kronfeld[1], T.M. Hess[1], J.E. Waldron[2], R.K. Splan[1], K.E. Saker[1], and P.A. Harris[3], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*Rectortown Equine Clinic, Rectortown, VA,* [3]*WALTHAM Centre for Pet Nutrition, Melton Mowbray, U.K.*

This study investigated if antioxidant supplements influenced antioxidant status and oxidative stress during endurance exercise. A pre-competition survey enabled 46 horses to be paired by nutrition and performance then randomly assigned to two groups. Three weeks prior to the competition, one group (E) was orally supplemented with 5,000 IU vitamin E/d, the other group (EC) with 5,000 IU vitamin E plus 7 g vitamin C/d. The ride covered 50 miles of terrain ranging from 400 to 1400 ft elevation. Blood samples, temperature and heart rate, were taken the day before the race (PRE), 13 and 35 miles during the ride, at completion (50 miles), and after 20 min of recovery (REC). Plasma lipid hydroperoxides (LPO), $\alpha$-tocopherol ($\alpha$-TOC), ascorbate (ASC), creatine kinase (CK), and aspartate aminotransferase (AST), erythrocyte

total glutathione (GSH) and glutathione peroxidase (GPx) were analyzed. Thirty-four horses completed the race with the remaining horses not finishing for reasons including lameness, metabolic problems, and rider option. Treatment, distance, and placing were evaluated by analysis of variance in a mixed model with repeated measures. Treatment and placing had no significant effect ($P > 0.05$). Heart rate, temperature, CK (PRE 228.5 ± 21 IU/L), AST (PRE 279.3 ± 11 IU/L), GPx (PRE 46.9 ± 1.5 $\mu$U/mg protein) and LPO (PRE 8.2 ± 1.0 $\mu$M) increased, where GSH (PRE 118.1 ± 4.9 $\mu$U/mg protein) decreased with distance ($P < 0.0001$). Linear regressions were found for plasma CK ($r = 0.25$; $P = 0.001$), and AST ($r = 0.33$; $P < 0.001$) on LPO. These regressions establish an association between muscle leakage and a cumulative index of oxidative stress. With the parameters measured no advantage was found for EC over E; however comparable studies have findings been inconsistent with these results.

**Key Words:** Glutathione, Lipid hydroperoxides, Vitamin E

**687 Endurance exercise: is potassium supplementation beneficial?.** T.M. Hess*[1], R.M. Hoffman[1], J.E. Waldron[2], P.M. Graham-Thiers[1], C.A. Williams[1], K. Greiwe-Crandell[1], D.S. Kronfeld[1], and P.A. Harris[3], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA*, [2]*Rectortown Equine Center, Rectortown, VA*, [3]*WALTHAM Centre for Pet Nutrition, Melton Mowbray, UK*.

Many clinical signs evaluated by veterinarians during endurance races are manifestations of increased neuromuscular excitability. The resting potential is mainly dependent on $K^+$ distribution across the cell membrane, so that neuromuscular excitability increases when $K^+$ is extruded from muscle cells during exercise. Plasma $[K^+]$ is increased during long exercise at speeds of 4 m/s or greater. Therefore we tested a K-free electrolyte mixture (EM-K) versus commercial mixtures rich in K in a 50-mile race. Of 46 riders, 22 used commercial mixtures (CON), 24 EM-K. Blood samples were taken from the jugular vein the day before, just prior to the start, immediately after each horse arrived at the veterinary checks at miles 13, 23, 35 and 50 and 20 minutes post race. Samples were kept on ice for less than 60 min before analysis for pH, carbon dioxide and oxygen, and electrolytes (Stat Profile M Blood Gas Electrolyte Analyzer, Nova, Waltham, MA). Effects of time (or distance), treatment (CON vs EM-K) and their interaction were evaluated by ANOVA in a mixed model with repeated measures applied to 34 horses that finished the race. During exercise, plasma $[H^+]$ increased and greater increases were associated with lower performance. Increases were also found in plasma concentrations of $Na^+$, $K^+$, $Mg^{++}$, lactate$^-$, and $HCO_3{}^-$; decreases in $Cl^-$, and $Ca^{++}$. Comparing EM-K to CON, plasma $[H^+]$ was 3% lower at 23 miles ($P=0.10$), 35 miles ($P=0.075$) and 50 miles ($P=0.056$) in the EM-K group. Similarly, plasma $[K^+]$ was 8% lower at 50 miles ($P=0.032$). Plasma $[Ca^{++}]$ was 4% higher at 13 miles ($P=0.046$). Plasma $[H^+]$ may be taken as an index of fatigue, so the lower plasma $[H^+]$ associated with EM-K may have contributed to better performance. Lower plasma $[K^+]$ and, to a lesser extent, higher plasma $[Ca^{++}]$ associated with use of EM-K should moderate increases in neuromuscular excitability and its clinical manifestations.

**Key Words:** Horses, Electrolytes, pH

**688 Plasma hydrogen ion and bicarbonate changes during repeated sprints in horses are influenced by dietary protein.** P.M. Graham-Thiers*[1], D.S. Kronfeld[2], and P.A. Harris[3], [1]*Virginia Intermont College, Bristol, VA*, [2]*Virginia Polytechnic Institute and State University, Blacksburg, VA*, [3]*WALTHAM Centre for Equine Nutrition and Care, Melton Mowbray, UK*.

Dietary protein is acidogenic. A low protein diet may moderate metabolic perturbations during strenuous exercise. Twelve Arabian horses were assigned to a 2x2 factorial design with 2 levels of protein (7.5% CP supplemented with .5% lysine and .3% threonine, LP or 14.5% CP, HP) and 2 levels of fat (0%, LF or 10%, HF). After 4 weeks accommodation, horses performed a repeated sprint test: 3 min walk at 1.5 m/s and no slope, 3 min at 1.5 m/s and 3.5 m/s with 6% slope, then 6 1-min sprints at 7 m/s separated by 3 min walks, concluding with 30 min walk at 1.5 m/s and no slope. Horses then completed eleven weeks of conditioning followed by a repeated sprint test with sprints at 10 m/s. Blood samples were taken at rest, in the last 15 sec of sprint 1,2 and 6 as well as at 5, 10, 20 and 30 min. of recovery. Samples were analyzed for lactate, pH (and $H^+$), $pCO_2$, $pO_2$, $Na^+$, $K^+$ and $Cl^-$. Bicarbonate and strong ion difference (SID) were calculated. Regression analysis was used to determine the contribution of SID, $pCO_2$ and Atot to changes in $H^+$ and $HCO_3{}^-$. In the first SET, $H^+$ was lower for LP groups ($p =.0056$) Regression analysis revealed that changes in $H^+$ were attributed mainly to changes in $pCO_2$ in both LP and HP groups ($r= .98$ and $.97$ respectively, $p<.0001$). Bicarbonate was not different between diets but changes were also correlated to $pCO_2$ ($r = .94$, $p = .0008$). In the second SET, $H^+$ was lower in the HFLP group ($p = .022$). The main contributor to changes in $H^+$ was changes in $pCO_2$ but Atot was also a contributor for the HFLP group ($r = .77$, $p = .021$). Bicarbonate levels were higher in the HFLP group ($p = .043$). An effect in the LP groups regardless of fat level appears to be $pCO_2$ (HFLP $r = .90$, $p = .003$ and LFLP $r =.91$, $p = .002$) however, Atot is also an influencing factor (HFLP $r = .92$, $p = .002$ and LFLP $r = .98$, $p = .0001$). For the HP groups, regardless of fat level, the effect of SID is significant (HFHP $r = .83$, $p = .011$ and LFHP $r =.73$, $p = .032$). Changes in $pCO_2$ appear to be the main controlling factors for changes in $H^+$ and $HCO_3$ during strenuous exercise. However, smaller changes in Atot for the LP groups and decreases in SID for the HP groups contribute to the decrease in acidosis during strenuous exercise for horses fed LP fortified diets.

**Key Words:** Protein, Acid-Base, Horses

**689 Effect of management practices and training on plasma $tCO_2$ concentration in horses.** K.H. McKeever, A.M. Szucsik, V.B. Balaskonis, C.L. Betros, C.F. Kearns, and K. Malinowski, *Rutgers University, New Brunswick, NJ, USA*.

Three experiments were performed to test the hypothesis that common management practices would alter plasma total carbon dioxide ($tCO_2$) concentrations in horses. In Study 1, a crossover design was used to test the effect of a placebo and 7 electrolyte supplements (Lyte-Now, Stress-Dex, Summer Games, Electroplex, Enduramax, Acculytes, Performn# Win) on plasma $[tCO_2]$ measured before and after a simulated race test (SRT). Ten unfit Standardbred mares completed a SRT on a treadmill (6% grade). During the SRT horses ran for 2 min at 4 m/s, 2 min at the speed previously shown to correspond to $VO_{2max}$, 2 min at 4 m/s. Blood was collected before electrolyte treatment (-4 hrs), 10 min prior to exercise, and at 0 min, 60 min, and 90 min post-exercise. Study 2 used a similar protocol to examine the effects of a control diet and three pelleted feed supplements (Drive, Omelene, Strategy) on plasma $[tCO_2]$. Study 3 examined the effects of training and simulated quarantine (2 d of detraining) by comparing resting plasma $[tCO_2]$ in samples collected from 10 unfit and 17 moderately trained (12 wks @ 60% HRmax) Standardbred mares. Mares were maintained on the same diet and feeding schedule and samples were obtained at the same time of day before exercise. In Study 1, there were no differences ($P>0.05$) in plasma $[tCO_2]$ across the 5 sampling intervals due to electrolyte treatment. In Study 2, there were no differences ($P>0.05$) across the 5 sampling intervals due to dietary supplement. In both studies, there were differences ($P<0.05$*) in plasma $[tCO_2]$ across sampling intervals (-4 hrs, -10 min, +0 min, +60 min, +90 min) that were attributable to acute exercise (mean ± SE; 34.4 ± 0.9; 33.2 ± 1.1; 20.2 ± 0.8*; 31.5 ± 0.8; 30.3 ± 1.6 mMol/L). In Study 3, resting plasma $[tCO_2]$ was lower ($P<0.05$) in trained (31.4 ± 1.9 mMol/L) vs. untrained horses (34.4 ± 0.9 mMol/L). There were no effects ($P>0.05$) due to detraining. It was concluded that acute and chronic exercise affect plasma $[tCO_2]$.

**Key Words:** Equine, Horse, Total carbon dioxide, Exercise