Kentucky Equine Research®

World Leaders In Equine Nutrition

CONFERENCE 2018

30 Years

OF PROGRESS IN EQUINE HEALTH AND NUTRITION

Notes

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OF PROGRESS IN EQUINE
HEALTH AND NUTRITION

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Welcome

Welcome to the Kentucky Equine Research Nutrition Conference. This year marks a milestone for Kentucky Equine Research, as it is our 30-year anniversary. We are glad that you have chosen to share in the celebration with us.

Since 1988, incredible advances have been made in not only how we nourish horses, which is our specialty, but also in how they are cared for by veterinarians. The world-renowned speakers we have gathered for this conference were asked to present a 30-year review in their areas of expertise, and each timeline showcases important advances in nutrition and veterinary care.

As you listen to the speakers and flip through these proceedings, you'll find the volume of knowledge that has been uncovered throughout the last three decades by these researchers and others is truly impressive. Enjoy the presentations!

We look forward to the exciting advancements on the horizon in each of these areas.

Thirty Years of Research

Joe D. Pagan, Ph.D.

As a researcher, I've found that more than one path can lead to a beneficial endpoint, especially when it comes to figuring out how best to feed horses. Many people think of research as the familiar "problem-solution" model. While this is true in some instances, it is not always the case. If not the familiar problem-solution model, what stimulates research? How do we come up with questions to answer?

Technology As Impetus

A point I've been thinking a lot about lately involves how horsemen and researchers use technology and how that technology has shaped research avenues. In some ways, technology completely retooled how horses are fed. Take, for example, nutrition of young horses.

In the mid-1980s, a landmark study was published by researchers from The Ohio State University in which a correlation was found between the level of copper in a breeding farm's ration and the incidence of metabolic bone disease in the farm's foals. Metabolic bone disease, later renamed developmental orthopedic disease (DOD), is a major problem for many horse breeders, and it causes huge economic losses due to lameness in performance horses.

Following this study, researchers around the world concentrated on finding the link between trace minerals—particularly copper and zinc—and bone development. This resulted in a research flare, or a burst of interest in a particular topic, in this case growth and bone disease. Out of this flare came important findings, including one from New Zealand that demonstrated trace mineral nutrition of the pregnant mare also affected subsequent bone health of her foal.

Since then, the feed industry has universally embraced the importance of trace mineral fortification for broodmare and foal feeds, and high levels of copper and zinc have become standard.

Despite this dietary change, DOD continued to show up, especially in precocious, fast-growing young horses. With the introduction of radiograph repositories at major yearling sales just over 20 years ago, it became more and more important for racehorses to have pathology-free X-rays at the time of the sales. When potential buyers can quantify the size and location of a lesion, it's often the death knell for an athletic prospect.

Thus, it became clear that DOD was caused by more than just an imbalance in trace minerals. In 2000, Kentucky Equine Research looked at the glycemic response of feeds and the incidence of osteochondritis dissecans (OCD), a particular form of DOD in which cartilage maturation is disrupted, failing to form normal bone. We found there was a correlation. If farms had a high-glycemic response, then the farms had a high level of OCD; farms with a low-glycemic response had a low incidence of OCD. Incidence correlated well with glycemic indices of the feeds.

Interestingly, the glycemic indices of the feeds were fairly narrow, and the percent of calories coming from carbohydrate was in the 40-50% range. When these data filtered into the mainstream, many feed manufacturers lowered carbohydrate calories to 30-40%. Some feed manufacturers went much lower, to 10%, for example. What happened? Breeders that followed the extreme low-carbohydrate recommendation ended up with a bunch of scrawny young horses with weak muscle definition. Like the young horses with DOD lesions, these too proved difficult to sell well.

This represents an example of another theme I have seen developing, and that is the idea of unsuspected problems cropping up when drastic changes are made to the way horses are fed. In a way, it's much like breeders being laser-focused on certain physical characteristics of show animals. Many times these characteristics eventually lead to problems because breeders take cosmetic features past the point of usefulness and attractiveness and into the realm of defect. Sometimes people go too far, and in the case of the poor-growing young horses, this is certainly true.

I don't do nearly as many presentations to breeding groups as I once did, but when I do, the talks I give today are not much different than the talks I gave 15 years ago. Why? There's not much new to talk about. No one is researching growth or DOD, or at least not as extensively as they were when it was a hot-button topic. For instance, I gave a few talks to British Thoroughbred breeders recently. The topics I selected included environmental effects of growth, showing how weather, pasture availability, and day length affect growth rate; growth rate differences in varying geographic regions; and characteristics of skeletal growth, but there was not much new about how to prevent DOD. In the late 1980s, that's all we talked about. How much copper? What form of copper? How does copper interact with other minerals? Now, people expect feeds to be formulated with appropriate amounts of cooper and zinc, and the topic has become passé.

I should touch on the use of ration balancers at this time. When there was realization that high-growth rates and high-starch might have an effect on DOD in growing horses, the idea of separating calories from the rest of the ration came about within the industry. Before this, it was always, "No, we must put energy sources and micronutrients together." While the importance of the micronutrients had been illustrated, it became apparent that the calories may or may not be necessary, based on the individual horse.

One of our innovations was MicroSteed, ration-evaluation software that we first started working on in the early 1990s. With MicroSteed, we could quickly see how well different components of a ration were meeting requirements. This is when balancers became popular. I remember distinctly that when we started to use balancers therapeutically for bone disorders, we would get pushback from veterinarians. Some nutrition consultants would say that this resistance continues to this day!

If, for example, a yearling has physitis, and the recommendation was to feed it two pounds of a balancer pellet, the vet would say, "That's 30% protein. You will blow the knees off of it. They can't have that much protein because protein is causing this problem." That's a widespread way of thinking to this day. I would try to explain that four pounds of 15% feed equals two pounds of 30% feed and six pounds of 15% feed provides more protein than two pounds of 30% feed, but I wasn't always convincing. Having a tool like MicroSteed, where you can get rid of the percent protein—what appears on the MicroSteed graph is percent required—you don't have to worry about the percent protein of the feed. Farm managers seemed to respond positively to the visual of an easy-to-read graph.

Balancer pellets were designed to be fed with cereal grain. The balancer was really a "grain balancer," not a "ration balancer." That name more clearly defines the intent behind that particular feedstuff. They started to be fed without grain to balance the entire ration rather than to balance just the deficiencies of grain.

Balancer pellets are certainly useful in some feeding situations, but then the ultra-balancer came into play, and that's the result of the obesity epidemic. We have gone to extremes to nourish these animals that have unusual requirements that are completely different than they should be. I will touch on obesity and metabolic disease later.

Another example of technology affecting research and nutritional standards involved the introduction of the flexible endoscope to veterinary medicine. This device allowed veterinarians and researchers to view the stomach. Prior to this, necropsies had revealed that some horses had lesions, but there had been no way to look inside the stomach of a living horse. What they found, not surprisingly, were varying degrees of ulceration in the stomach, so a research flare ensued, with several scientists investigating gastric health. Research led to the development of products designed to keep ulcers from forming. Many of these were over-the-counter products that buffered stomach acid or coated the lining of the stomach. Had the endoscope not been introduced, horse owners wouldn't have been worried about the health of their horses' stomachs.

Side-Effects of Treatment

Omeprazole was the first FDA-approved medication available for curing gastric ulcers in horses. The medication was heralded by horse owners and veterinarians alike. Once gastric ulcers are cleared with omeprazole, management changes can be made to keep new ulcers from forming. Use of omeprazole in certain horse populations is extensive (racehorses, high-performance horses, high-strung horses), sometimes daily, so now we are studying the effects of omeprazole on nutrient digestibility. Results of this research suggest that omeprazole has an effect on calcium digestibility. In essence, we changed the calcium requirements of horses by treating gastric ulcers. Are we trading one problem for another?

This same idea of changing requirements comes to mind when thinking of the widespread use of furosemide, or Lasix, in racehorses and certain other performance horses, like barrel-racing horses. Through studies we conducted at Kentucky Equine Research, we know that furosemide inhibits the ability of the horse to absorb sodium and chloride, leading to large urinary losses of these electrolytes and calcium. By administering a medication meant to prevent bleeding, or exercise-induced pulmonary hemorrhage, we inadvertently caused a change in electrolyte and calcium requirements. Fortunately, we were able to develop an electrolyte replacement product (Race Recovery) that corrected these imbalances.

Another example involves low-starch diets. Many horses require low-starch diets to perform optimally. This is the case, for example, with horses with certain myopathies. In the other paper I assembled for this conference, I recount how the first low-starch feed (Re-Leve from Kentucky Equine Research) came about and eventually marketed to horse owners. The feed came to fruition through a partnership with Dr. Stephanie Valberg. Her knowledge of myopathies paired well with what we knew about nutrition and feeding high-performance horses. Eventually, we landed on the notion that these excitable Thoroughbreds prone to tying-up were much more docile on high-fat, high-fiber, low-starch feeds, and this calmness led to a decrease in the incidence of myopathy.

As happens with these things, the information trickled into other segments of the performance-horse world. If it's nutritionally sound for racehorses, it must be fine for other horses, so let's feed it to all horses, right? Now, a new problem might be brewing. Could horses be developing muscle problems because they're being fed high-fat, low-carbohydrate diets?

This is the basis for some new research that we're planning with Dr. Valberg's group. Our hypothesis is that endurance horses and Warmbloods with certain types of myopathies have deficiencies in their ability to keep their antioxidant status up to snuff and this may have a genetic basis. The only time it's really a problem is when excessive oxidative stress occurs, when not having adequate antioxidants can potentially cause muscle damage. One trigger factor may involve high-fat diets. A high level of fat metabolism produces reactive oxygen species (ROS) which may lead to oxidative stress.

Novel Solution to a Well-Known Problem

Returning to the theme of gastrointestinal tract health, equine nutritionists understood a long time ago that hindgut acidosis was likely in certain horses, especially those with high grain (racehorses, three-day-event horses, etc.) and high fructan intakes (grazing horses). Buffering acid in the rumen of ruminants is standard operating procedure, but no one tackled the problem in the horse, likely because of the anatomical differences between horses and cattle.

I was visited one day by a representative from a company that specializes in encapsulation technology. As we talked, I asked if he could come up with a way for us to deliver sodium bicarbonate to the hindgut of the horse. It turned out that the company was already encapsulating sodium bicarbonate for the food industry, so it became a matter of me providing the dissociation characteristics required for the product to work in the hindgut of horses. The company went back to its lab, adjusted the level of protection afforded by the encapsulation material through some in vitro work, and came back to us with a prototype.

We then tested it with a high-grain feeding study to assess how well the product buffered the hindgut of exercised Thoroughbreds. Once we realized we had strong data, we introduced EquiShure, a hindgut buffer, to the marketplace.

Present-Day Focus

When I started conducting equine nutrition research in the 1980s and 1990s, there was a lot of interest in orthopedic disease and nutrition of the performance horse, leading to multiple research flares and some great research by several scientists. More recently, this has morphed into the explosion of interest in the obese, metabolic horse. Fewer and fewer papers presented at meetings and published in peer-reviewed journals pertain to the high-performance horse. It seems everyone's interest has moved away from improving athleticism in performance horses to how to keep fat, sedentary horses healthy. That, for me, is a depressing change in the focus of much research. The demographic of the horses dictates this, though, as do the horse owners and the funding sources.

I think there are many more fat horses around than there used to be. I don't remember in 1980s or 1990s being concerned about obesity. We knew there were laminitic horses, of course, but the whole concept of insulin resistance was a new revelation. This gained popularity with the studies performed by Dr. David Kronfeld and his team at Virginia Tech.

I think one of our most classic studies at Kentucky Equine Research involved this subject. We used older, overweight Thoroughbreds that were not necessarily metabolic, and we fed them one of two isocaloric diets—a high-fat diet or a traditional diet with starch. The horses became glucose intolerant on the high-fat diet, but they became normal again when we added a couple of ounces of fish oil (EO-3) to their daily ration. That's an interesting finding, and it's one that we've never really packaged properly for the end user. Now, there are lots of well-meaning horse owners who are feeding high-fat diets thinking they're improving glucose intolerance, not making it worse. I think if that were more well publicized—that feeding high-fat diets is not without risk at times—I think owners would revisit traditional feeds for healthy horses. If owners have a legitimate concern about the carbohydrates, at least feed horses on low-starch, high-fat diets marine-derived omega-3s.

Future of Equine Nutrition Research

The latest flare involves nutrigenomics and nutrigenetics. Nutrigenomics is defined as the role of nutrients in gene expression, and nutrigenetics is the effect of genetic variation on dietary response. The classic concept of nutrition is that a nutrient is fed to be a substrate for a protein, a source of energy, a cofactor for an enzyme system, or any other physiological process, as opposed to feeding something to upregulate or downregulate genetic expression of a set of genes. That really changes the entire concept of excess and deficiency. We have begun some work in this area with Dr. Kristine Urschel from the University of Kentucky as a collaborator. ✦

Advances in the Understanding of Colic

Nat White, D.V.M. , M.S., Diplomate A.C.V.S.
Professor Emeritus of Equine Surgery

For background, why did you choose to study colic? What attracted you to this specific field of study? How did you wind up being an authority on this health problem?

During August of 1972, 15 horses with colic were admitted to the University of California, Davis Teaching Hospital for surgery. Fourteen died or were euthanized. The fifteenth, which survived surgery for an enterolith, returned two months later with fatal liver disease. I thought there had to be a better way to save horses with severe intestinal disease. My experience as a surgeon initiated my entry into research aimed at understanding the diseases causing colic. Much of the knowledge I attained was from seeing as many cases as possible and garnering information from each case. I am convinced my efforts and that of many others in both research and education have improved the outcome for horses over the last four decades.

When did you first begin to study or research colic in particular? At that time, what were the primary research interests in the field? What was happening?

My first venture into research on colic was taking ECGs on horses suffering from colic at Davis. The changes seen in horses in shock indicated a direct effect on the heart. Subsequently during two years at Kansas State University while studying pathology, examination of intestine combined with training in electron microscopy set up the opportunity to investigate the ultrastructural changes during intestinal ischemia.

This became a primary focus for research due to the poor outcome for horses suffering from intestinal strangulation. The serious effort to investigate the effect of intestinal ischemia started with Dr. Jim Moore, then at the University of Missouri. We worked on a project that demonstrated the effect of reperfusion injury, which helped to explain the continued intestinal degeneration after what we hoped was a successful surgery. The results stimulated both of us to seek resources to complete more research. This also resulted in creation of a colic research team at the University of Georgia. During the subsequent decades the research focus has expanded to include epidemiology, endotoxic shock, diagnostic tests, surgical technique, critical care, and evaluation of treatments.

In those early years, what was considered state-of-the-art technology when it came to diagnosing colic? Was diagnosis based solely on clinical signs? What were those signs?

My role as an intern at Davis was to work up colic cases and attempt to make a diagnosis. Clinical signs consisting of heart rate, auscultation, evaluation of stomach reflux, and rectal examination were the primary diagnostic tools, as they are today. Lab work was restricted to PCV, total protein, and CBC. Blood gas, electrolytes, and liver enzymes were not initially evaluated but could be helpful after treatment was initiated. We often had to wait until the next day to get these types of laboratory tests completed.

Evaluation of peritoneal fluid was just starting. At first, we often waited for the fluid to become serosanguineous before diagnosing a strangulating lesion and recommending surgery. Now the need for surgery is more often based on pain and physical signs when peritoneal fluid may be normal. We also started using serum lactate to provide a prognosis, but it was not easily obtained for timely evaluation. Lactate is still a key component of the evaluation including measuring lactate in peritoneal fluid. The addition of abdominal ultrasound has been a major improvement in the ability to make an early diagnosis.

In those days, what were the standard treatments for different types of colic?

Treatments in the 1970s consisted of mineral oil, pain relief (with phenylbutazone, choral hydrate, and dipyrone), and surgery. Surgery became more successful after the introduction of halothane anesthesia, which helped the recovery compared to the results with intravenous anesthesia. Administration of large volumes of a balanced electrolyte solution had just started to be used in the early 1970s and was responsible for improved outcome.

The introduction of flunixin meglumine in the late 1970s replaced other treatments and became the primary drug for treating colic and endotoxic shock. Because it is a potent analgesic, we had to learn how not to overuse it. Flunixin meglumine is still a primary treatment for colic, and evaluating the horse's response to its initial administration is now an important way to determine the severity of intestinal disease. Treatments are now much more focused–based on making a diagnosis. However, for simple colic, analgesia and hydration are still the primary treatments.

In looking specifically at the work you've done, what has changed in the way you study colic? Specifically, how has technology paved the way to more accurate diagnosis?

Much of my research was directed at trying to find a way to reverse the intestinal injury caused by ischemia and reperfusion. Application of electron microscopy, use of intestinal blood flow monitoring, and evaluation of the effect of neutrophils and capillary leakage in ischemic intestine led to a new understanding of the changes occurring during colic. The more recent ability to evaluate release of cytokines and inflammatory mediators has changed our understanding of the events that occur during colic. That said, making a diagnosis remains a challenge due to the similar clinical signs created by a variety of diseases. Use of ultrasound is most likely one of the most important technology advances to help make a diagnosis. Rapid horse-side tests are becoming available and specifically helps for rapid assessment of lactate.

Your accomplishments in understanding, researching, and reporting on colic cannot be undervalued. What do you feel are your greatest achievements in this area?

My research has all be aimed at trying to understand the abnormalities causing gastrointestinal disturbances and the effects on the horse. I think my research on intestinal ischemia and colon hydration has helped to save horses. However, education of veterinarians and horse owners about how to make a rapid assessment to categorize the type of disease may be my greatest contribution. In particular, training of residents and graduate students to continue research in my areas has been rewarding.

What work have other researchers done in this area that you feel will have a lasting impact?

Epidemiological studies have helped define disease severity, prognosis, and causality. Studies detailing the effects of endotoxin have improved treatment. New research is increasing the understanding of the role of the intestine and its microbiota in maintaining an equilibrium in the digestive tract. Characterization of the intestinal response to ischemia and inflammation with an understanding of role of the neutrophil and endothelial cells in the cascade of events has improved assessing intestinal viability and the systemic response to intestinal injury.

How do you think other researchers will approach colic in the future?

Three areas deserve more research. First is more prospective epidemiologic studies are needed to understand the relationship of the environment and diet to mild colic, which represents more than 85 percent of colic in horses. Second is nutrition and understanding the microbiome in gastrointestinal health and function. New technology is allowing rapid identification of the flora in the horse's intestinal tract in varied conditions. Third is use of stem cell research to define the response of the intestinal cells to insults and potentially to stimulate regeneration in injured intestine.

The technology for genetic testing will also help to decide if there is a genetic predisposition to intestinal dysfunction. When combined with analysis of the microbiome, there may finally be answers for some of the questions about the cause of the most common type of colic: the simple colic with no specific diagnosis.

During your career, what other areas of study were you interested in? Were you able to pursue those as thoroughly as you did colic?

I was interested in pursuing research for the problems I saw every day in the hospital. Musculoskeletal problems including tendon and ligament disease, joint disease, muscle problems, and foot disease. Though interested in a number clinical problems, I was not able to complete in-depth research in all these areas, but did complete clinical studies, cases series, and defined new techniques and treatments. My goal has been to increase the knowledge in equine medicine and surgery to improve outcomes for horses and their owners. We should not underestimate the value of trying new techniques or treatments on individual patients. In some cases stepping away from what has been done before can lead to a medical breakthrough. That said, observational research must be carefully evaluated. One or two observed responses to a treatment often is caused by chance.

Instead of an exhaustive reference list, can you suggest two or three review papers or book chapters that would provide an overview of the subject? Feel free to suggest something you've written or co-written. Alternatively, could you suggest a couple milestone studies in this area?

I am partial to this book: *The Equine Acute Abdomen*, Third Edition. 2017. Ed. A. Blikslager, N. White, J. Moore, and T. Mair. Wiley-Blackwell.

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What else might be interesting to horse owners and veterinarians?

There is an old saying: "As long as there are horses there will be colic." Colic remains a mystery and a challenge. However, it is important not to accept that diseases causing colic are unsolvable. Solving the mystery requires research and research requires financial support. Currently there are not enough research dollars to sustain the in-depth research programs needed to investigate the diseases affecting the horse's gastrointestinal tract. Educating the horse owner about the need for financial support is critical for a future with less equine colic. ✦

Three Decades:

The State of Energy in Horse Feeds

Joe D. Pagan, Ph.D.

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Can you tell us a little about your interest in energy as it relates to equine nutrition?

My interest in energetics dates back to my days as a student. After graduating from the University of Arkansas with a bachelor's degree, I went to Cornell University for a master's degree, and eventually a doctorate. I studied under Dr. Harold Hintz—we knew him as Skip—and he allowed me to pursue my interest in energy requirements of the performance horse. This was the early 1980s, and none of the universities had high-speed treadmills at that time, so we improvised.

I borrowed a tractor and an old hay wagon. We loaded all of the equipment onto the wagon, connected the horses to a calorimeter, ran them around a half-mile Standardbred track, and measured how much oxygen they consumed and how much carbon dioxide they produced. We were also able to measure how much energy the horse consumed and what fuel they were burning.

Looking back, the entire setup was equal parts ingenious and crazy, but this was my introduction to exercise physiology, and results from this formed the energy requirements for performance horses. That's old news now, even though that research was cited in the latest edition of *Nutrient Requirements of Horses*.

When I finished at Cornell, I thought I needed to up-spec things a bit, and I wanted to go where there was a high-speed treadmill. I traveled to Sweden for some post-doc work, because the vet school there had a treadmill. We could make these measurements without chasing horses around a racetrack. The researchers there were also able to take muscle biopsies, so we could examine actual muscle tissue to see exactly what the horse was using for fuel.

Before we talk about some of the innovations in feeding performance horses, in particular energetics, what did horse-feeding look like prior to the advent of commercial horse feeds?

As herbivores, horses are content to wander and consume plant fiber. They have digestive tracts designed to process huge volumes of forage nearly continuously. Once domesticated, horses were used in different ways, usually made to work hard, and they could not meet their energy requirements on forage alone. Today, we think of energy requirements as the horse's ability to perform the work asked of it and to maintain appropriate body condition for the sport in which it is engaged. Back then, energy requirements probably meant the ability to put in a full day's work without shutting down. To support this kind of work, cereal grains became staples in diets.

Cereal grains are actually a rich source of starch, and they have different levels of starch: corn has the highest, barley has intermediate, and oats has the lowest. While starch is useful for many horses, it comes with its fair share of hurdles.

When did you start looking at other ways to provide horses with energy aside from carbohydrates?

The work in Sweden was my first dive into looking at alternative energy. We investigated a few different energy sources, and one of the most interesting ones was fat.

Fat became a popular alternate energy source in the 1980s. Adding fat to feeds was really the first time feeding performance horses changed significantly; before that, it was just carbohydrates. Fat has a lot going for it, including energy density. It has two and a half times as much energy as corn and three times as much energy as oats, so we can pack a sizeable punch with fat. Horses can digest fat pretty well, and fat proved palatable. One of the most significant research efforts during the 1980s concerning fat came from Texas A&M University, where the team there was feeding beef tallow to horses.

A few years later, after starting Kentucky Equine Research, we did a lot of work with feeding fat. We found we could teach muscles to burn fat, so that other fuels, like glycogen, could be spared. This was an especially important finding for endurance athletes.

What other energy sources popped up after the introduction of fat?

We began looking for other sources of fuel for performance horses not long after fat came into the spotlight. We came upon beet pulp in the 1990s, which turned out to be a great alternative energy source for horses. We coined the term "super fiber" to describe beet pulp because it contains fiber that is extremely digestible, nearly twice as digestible as fiber found in normal hay.

We then realized that we could use multiple energy sources in a single feed. During the 1990s, the popular performance horse feed became one that had a mix of energy sources. We used cereal grains as a base and then added fat (vegetable oil, rice bran) and fermentable fiber (beet pulp, soy hulls). To this day, this is the most popular type of feed for racehorses.

What researchers were prominent at this point?

As I mentioned, the Texas A&M University program, headed by Dr. Gary Potter, had some funding that allowed his group to work with feeding fat to horses. Another influential researcher was Dr. David Kronfeld, who was at Virginia Tech. Kronfeld was a very charismatic, opinionated guy, and he had a ton of graduate students. He went out and really campaigned the idea that fat- and fiber-rich feeds were beneficial to all types of horses.

At the time, the university-based graduate programs had lots of graduate students, and the students at a university would often work as a cohesive group on the same idea. If, for example, Kronfeld was interested in studying fat, then much of his team studied fat. This concentrated effort led to some important findings. The value of some of these graduate programs, like Potter's and Kronfeld's, should not be underappreciated. When you look around now at the researchers in academia, a lot of them came through Kronfeld's program. Out West, a lot of them came through Potter's program.

What else happened in the 1990s that changed how energy was delivered to horses?

A huge change came in the mid-1990s, and it came through our collaboration with Dr. Stephanie Valberg, a veterinary professor who was at the University of Minnesota at the time. She is a specialist in muscle physiology and muscle disease. Stephanie was working on her doctorate in Sweden at the same time I was there working on my post-doc.

Stephanie and I have collaborated on research throughout our careers, and that continues to this day. At that time, in the mid-1990s, she was interested in some very specific muscle disorders, and she asked for our help to see if there was a nutritional connection. The types of muscle disorders that she was particularly interested in were forms of tying-up or exertional rhabdomyolysis.

We know now there are several forms of the disease. The one that we were most interested in at the time was called recurrent exertional rhabdomyolysis or RER. This form was found in racehorses, especially Thoroughbreds and Standardbreds.

Stephanie found that the disorder lies in the ability of the horse to regulate the release of calcium in its muscle to make it contract and then relax. These horses have normal insulin sensitivity, and they don't have abnormal polysaccharides. We know in Thoroughbreds it is more common in females than males. About 5% of racing Thoroughbreds have it, and there is a common behavioral characteristic: they have a nervous temperament. Typically, these horses are exercised pretty hard, and you have to feed them quite a bit of feed to fuel performance and maintain body condition. Affected horses usually have these episodes when they're getting really fit, when there's an uptick in their training. As they become fit, they become more excitable. Resting creatine kinase, a measure of a muscle enzyme, is normal. After they tie-up, though, the amount of creatine kinase in the blood increases dramatically. We also know that lack of routine daily exercise and high-starch diets make it worse.

Stephanie started to do some pioneering work looking at the genetics of tying-up. She found that there are several prominent Thoroughbred family lines that have offspring with RER. They did breeding trials that confirmed that the disease is genetic. But, it doesn't happen in every horse. That was the mystery: what was the trigger? She came to us and said, "Well, if high starch causes it, could the trigger factor actually be the diet that the horses are eating?" We got together with Stephanie, and did the first of many studies on that topic.

In the end, we created a completely different type of performance horse feed. We took out all of the grain and used nothing but fat and fermentable fiber. We got the nonstructural carbohydrates (NSCs) down to 10%; we took away most of the starch and sugar and replaced it with these other types of energy. As a point of reference, typical performance feeds of that time derived 40-45% of their calories from NSCs, so this change was radical.

We tested these feeds on some horses that we knew had a genetic predisposition to tying-up and had actual problems with tying-up previously. When we kept these horses on low intakes of traditional performance feeds, they didn't tie-up, even after a standardized exercise test in which we tried to bring about a tying-up episode. If we raised their intake of traditional grain to the caloric intake they needed to maintain their body weight, the horses tied-up and had concomitant increases in circulating creatine kinase. When we fed the same number of calories with Re-Leve, the new low-starch feed we had created, we couldn't get them to tie-up. We were able to prevent the clinical expression of tying-up in these horses by altering the source of energy. That was a pretty big deal!

Why did this work?

In Stephanie's subsequent trials, we found that the feed works not because of the change in muscle glycogen, and not because of the old-wives' tale that these horses have high levels of lactic acid. She measured lactic acid in horses that tie-up, and the lactic acid is no higher. Tying-up is not affected by dietary cation-anion balance, nor does it have anything to do with calcium intake.

This type of feed works because the horses are calmer. During the research trials, the horses had a quieter demeanor during standardized exercise tests. Before we started exercise, they had lower heart rates. So it seems then, in the end, what we did was change behavior by the type of energy we fed.

Stephanie is way down the pike in terms of trying to figure out a genetic basis for this. There are multiple genes involved. Why was it more prevalent in fillies instead of colts? Is there another gene that occurs more often in fillies than colts that's a trigger gene? Is there an anxiety gene in these horses? She's closer than anyone to figuring out these puzzles.

The creation of the really low-carbohydrate feeds literally changed the way that we look at feeding sport horses today. Modern sport horse nutrition is really a balancing act. When we are trying to feed a horse, we're trying to get maximal athletic performance but, at the same time, it's a balance against behavior. We didn't used to worry about that 20 or 30 years ago. Is it because we know more about different energy sources? Are the horses different or are we riding a different type of horse today than before? Or are the riders different than they were 20 or 30 years ago?

To that point, not all horses are the same and not all horses are asked to do the same kind of work. Can you touch base on that in regard to energy?

Just as cars need different levels of octane, horses need different levels of octane. Some horses run well on diesel fuel, and some need high-performance fuels. So, we make different types of feeds. That's the reason why there is more than one performance feed in the marketplace.

Kentucky Equine Research has designed different performance products for many feed manufacturers, and across those feed lines we often have a low-starch version and then feeds that have progressively more starch. All of those feeds are appropriate for performance horses, but all are not suitable for every individual performance horse. The challenge is in figuring out which feed is best for the individual horse.

Glycogen is the primary fuel used by horse muscle, so in nourishing performance horses, we need to make sure that we offer feeds that can replete muscle glycogen. The amount of muscle glycogen that's used during exercise starts out very low. As the horse engages in higher speeds, it starts to rely upon anaerobic metabolism, and muscle glycogen utilization skyrockets. Glycogen is used very inefficiently, and it's used up quickly.

What does that mean for different types of performance horses? What does it mean for an endurance horse versus a driving horse? We don't know for sure in some of these horses. For instance, we don't know how much muscle glycogen is used in jumping. Certainly a lot is used.

We don't know how much extra muscle glycogen is used in driving. Quite a bit, I'd say. We know a little bit more about horses that go for longer amounts of time. For instance, endurance horses race in a speed range between 250 and 500 meters per minute, depending upon where it is in the course of a 100-mile race. The amount of muscle glycogen that is being used is actually pretty small at that point. But, they go a long, long time. Fortunately, as I mentioned previously, endurance horses know how to switch fuel and begin using fat. They can change the fuel tank and preserve glycogen.

If we look specifically at three-day-event horses, glycogen burn becomes greater. As you go into higher and higher levels of eventing, and cross-country gets faster and faster, the burn gets higher. The amount of muscle glycogen that is actually used is going to matter to a certain degree on what level you are actually competing. And again, we have to throw in a little extra for all of the jumping efforts.

Switch to Thoroughbred racing, though, and you're in a completely different ballgame. Thoroughbred racing is done at speed. In training and in racing, they use massive amounts of muscle glycogen. They don't run very long, but when they're running, they burn a lot of muscle glycogen. We have to make sure that we replete that muscle glycogen.

We did a trial a few years ago with Stephanie. One of the tools that we use extensively is the muscle biopsy, which allows us to figure out how much muscle glycogen is actually burned by a horse. In this study, we measured muscle glycogen. We exercised the horses hard enough to deplete 30% of their muscle glycogen, which is quite a bit. We then fed them for three days afterwards with either a high-NSC feed or a no-NSC feed. We found the horses repleted muscle glycogen when they had some carbohydrates in their diet, but they could not replete muscle glycogen when they did not have any carbohydrates in their diet. If you've got a horse that's exercising hard enough to utilize muscle glycogen, you need to make sure you feed something that can replete that muscle glycogen. This research trial solidified the idea that you have to match the right horses with the right feed.

For readers that want to know more about your work, what would you suggest them to read?

The work I did at Cornell was published in the *Journal of Animal Science,* back in 1986. Like I mentioned previously, it's the foundation for many energy recommendations that stand today. What's more, the second paper even has some sketches of the calorimeter I used to measure energy expenditure during exercise.

Pagan, J.D., and H.F. Hintz. 1986. Equine energetics. I. Relationship between body weight and energy requirements in horses. 63:815-821.

Pagan, J.D., and H.F. Hintz. 1986. Equine energetics. II. Energy expenditure in horses during submaximal exercise. 63:822-830.

The other work, including the collaborative work with Stephanie Valberg, has been reported in the three volumes of *Advances in Equine Nutrition* and elsewhere, all of which are available online at ker.com. ✦

The Changing Equine Silhouette:

Equine Metabolic Syndrome Through the Decades

Pat Harris, M.A., Ph.D., Vet.M.B., M.R.C.V.S.

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Can you tell us a little about your background?

Whilst undertaking the house physician's job at Cambridge University Veterinary School and thinking of continuing an academic career, I decided to look for clinically relevant doctorate positions. One came up at the Animal Health Trust, in Newmarket, that was a joint position between the clinical and the physiology departments looking at various aspects of the equine rhabdomyolysis syndrome (tying-up), an important metabolic condition. The doctoral programme was wide-ranging in its scope and included a large nutrition component, which fascinated me. Whilst investigating various aspects of different metabolic diseases as a post-doctoral researcher, I expanded my knowledge of equine nutrition and in 1995 joined WALTHAM and MARS to head up the equine nutrition-associated research programme. I often state that I am a veterinarian by training and a nutritionist by choice!

Give us a little history about metabolic diseases in horses and EMS in particular.

Metabolic diseases have been recognised for many years. Aristotle, for example, apparently referred to laminitis around 350 B.C. as "barley disease," presumably because it was associated even then with excessive consumption of grain. The first full description or review was possibly commissioned by Constantine the Great in the fourth century, although it was not published until A.D. 900. This noted, as we do today, that the condition, referred to as "gout" as well as "barley disease," could have multiple causes, including travelling on hard surfaces, overeating, and drinking too much cold water when overheated. Interestingly, considering today's link between obesity and laminitis, this review apparently recommended mild bleeding, exercise, and diet restriction as part of the treatment and management regimen.

The link between obesity and laminitis has perhaps been recognised for many centuries, but it was only in the 1980s that the potential link with insulin was recognised when an alternative explanation was given (Coffman and Colles, 1983; Jeffcott et al., 1986) for obese ponies being more susceptible to laminitis other than their excessive weight—that is, obese ponies were less insulin sensitive and therefore there could be a link between insulin resistance and laminitis.

This whole area was developed by Johnson and colleagues as well as the researchers at Virginia Tech led by Dr. David Kronfeld in the early 2000s (Johnson, 2002; Hoffman et al., 2003; Treiber et al., 2005, 2006), which led to the terms pre-laminitic metabolic syndrome, peripheral Cushing's syndrome, and equine metabolic syndrome (EMS). Australian researchers (Asplin et al., 2007; De Laat et al., 2010) then showed that laminitis could be induced in ponies and horses through maintaining a high plasma insulin concentration. For both studies, the mean insulin was around 1,000 µIU/mL and, interestingly, the pathology was different than that of other forms of laminitis, with stretching of the lamellar epithelial cells occurring within six hours of the hyperinsulinaemia (Patterson-Kane et al., 2018).

The term "equine metabolic syndrome" (EMS) became more commonly used, although this was disputed, especially by Kronfeld and coworkers (2005). In 2010, an ACVIM consensus statement by Dr. Nicholas Frank and international colleagues recommended the use of EMS to describe the phenotype of an animal with obesity and insulin resistance with a predisposition towards laminitis (Frank et al., 2010). The term was adopted in part because of the similarities with the human metabolic syndrome that was a collection of risk factors believed to predict the occurrence of coronary artery disease and type 2 diabetes mellitus in people. The panel of experts proposed that the EMS phenotype for the majority of affected equids should include:

- Increased adiposity in specific locations (regional adiposity) or generally (obesity);
- Insulin resistance characterized by hyperinsulinemia or abnormal glycemic and insulinemic responses to oral or IV glucose and/or insulin challenges;
- Clinical or subclinical laminitis that has developed in the absence of recognised causes such as grain overload, colic, colitis, or retained placenta.

The panel recognised that whilst "obesity is observed in the majority of cases, some affected equids have a leaner overall body condition and regional adiposity and others are normal in appearance." They also recognised a variety of other hormone and systemic markers that might be linked with EMS and the risk of laminitis.

What has changed more recently?

A great deal of work has been undertaken and reported in the period between this initial consensus statement and today (Durham et al., 2018; Rendle et al., 2018). This work has, for instance, suggested that horses with a body condition score of >7/9 should be considered obese (Dugdale et al., 2011a, 2012). Other work has shown that not all obese animals are insulin resistant—whether obese animals are insulin resistant or not may depend, at least in part, on the diet fed to induce the obesity (Bamford et al., 2016a,b)—and suggested that the risk of laminitis is a complex interaction between genetics and the environment (McCue et al., 2015). In fact, their response to an oral glucose/sugar may be one of the most important predictors of laminitis risk (Meier et al., 2018) rather than tissue insulin resistance per se, leading to the increased use of the term insulin dysregulation (Frank and Tadros, 2014) defined as any combination of fasting hyperinsulinaemia, postprandial hyperinsulinaemia (response to oral sugar test or consumed feeds), or tissue insulin resistance.

This led to a subtle shift in the definition of EMS in 2016, as defined by the Equine Endocrinology Group led by Frank and including many of those in the original consensus statement, to "a clinical syndrome associated with an increased risk of laminitis that includes insulin dysregulation and any combination of increased or regional adiposity, weight loss resistance, dyslipidemia, and altered adipokine concentrations."

More recently there has been increased acceptance that lean animals may retain the EMS phenotype and therefore an increased risk of laminitis, which has led to the most recent consensus definition of EMS by the Equine Endocrinology Group, proposing to exclude increased or regional adiposity as being a core component of EMS—that is, obese animals may or may not be EMS animals, and lean animals may or may not be EMS animals. Therefore, the key consistent feature of EMS being the presence of insulin dysregulation (ID) associated with an increased risk of laminitis, with or without other metabolic alterations and/or obesity.

Where does this leave us?

First, equine obesity (defined here as the excessive or pathological accumulation of fat) is commonly found, especially in certain native breeds, and remains a major welfare issue due to a variety of associated adverse consequences, including orthopaedic disease, hyperlipaemia, hyperthermia, infertility, and poor performance (Geor and Harris, 2013; Rendle et al., 2018). In general, it appears that body fat is likely to be greater than 20-25% of body mass in animals with BCS ≥ 7, and values of up to 47% have been measured in ponies (Dugdale et al., 2011a,b).

A BEVA-EBM study on pasture-associated laminitis concluded that "overweight animals that develop laminitis tend to have more severe signs than those of optimal weight. When laminitis does occur, overweight animals are more likely to die of the disease than their thinner counterparts" (BEVA Newsletter, 2009). Recent gain in weight has been shown to be a risk factor for laminitis (Wylie et al., 2013), and obesity also remains a risk factor for insulin dysregulation; therefore, obesity remains implicated in laminitis risk regardless of whether it is included in the core definition of EMS or not.

Second, if this definition is generally accepted going forward, EMS can only be diagnosed by demonstrating ID in the form of hyperinsulinaemia or abnormal insulin responses to oral or intravenous challenges.

Third, it is important to realise that by definition all animals with EMS will be at increased risk of laminitis but for genetic, management, and environmental reasons, they may not all go onto develop clinical laminitis.

Finally, the value of recognising EMS is to enable individual animals with an increased risk of developing laminitis to be identified and to allow appropriate prevention strategies to be put into place.

Explain the changing face of EMS diagnosis.

From the information provided above, it becomes obvious that it is not possible to look at an individual animal and say that it has or does not have EMS, although practically the diagnosis of EMS will often be assumed when there is obesity and laminitis and the laminitis cannot be explained by other causes (Rendle et al., 2018). Managing animals that are obese whether they have laminitis or not is necessary and should be implemented regardless of the results of any diagnostic test.

However, often diagnostic testing may be a very useful adjunct especially when discussing, for example, the risk of returning a laminitis-prone animal to pasture. Importantly, the value of using more dynamic tests is increasingly being recognised and work is ongoing to refine the protocols and the doses to use (Smith et al., 2016; Knowles et al., 2017; Jocelyn et al., 2018).

Increasingly, all the work being undertaken globally has meant that we have realised that the interpretation of any results needs to take into account dose, timing and nature of any recent feeding, potential stressors, laboratory method used, season, breed, test-specific reference range, etc. Insulin concentrations measured using one type of assay, for example, may not be the same as found with another; results of the oral sugar test can vary considerably even in the same individual and therefore testing conditions need to be consistent and only appropriately large changes should be considered clinically relevant. Further information on current recommendations can be obtained from the ECEIM 2018 consensus statement (Durham et al., 2018) and a recent current-perspectives roundtable publication (Rendle et al., 2018).

Do we have a problem of obesity in our horses? If so, why?

Absolutely. Obesity in horses is increasingly being recognised as a globally important welfare issue. The 1998 USDA National Animal Health Monitoring System (NAHMS) study estimated that approximately 1.5% of the horse population in the United States was overweight or obese. However, these estimates were based on owner assessment of body condition, which we appreciate likely underestimate actual body condition (Morrison et al., 2017a).

Indeed, the results of recent prospective studies in which more standardized methods were used for physical assessment have demonstrated that the prevalence of overweight/obesity in pleasure-horse populations is far higher than the NAHMS estimates. Wyse et al. (2008) examined 319 pleasure riding horses kept at 22 horse operations in southwest Scotland and found that 112 animals (35%) were fat (defined as BCS 5/6) and 32 horses (10%) were obese (BCS 6/6). In a study of 366 horses in North Carolina, 48% were considered to be overweight or obese (BCS >6/9, Henneke scale) (Pratt-Phillips et al., 2010), while a cross-sectional study of 300 mature horses in southwestern Virginia reported that 97 horses (32.3%) were (as the authors described them) overconditioned (BCS 7) and 56 (18.7%) were obese (BCS 8–9) (Thatcher et al., 2011). These studies were conducted during the summer months when horses may be more predisposed to weight gain due to increased availability of pasture forage and seasonal changes in feed intake and metabolism.

In a small survey study (127 horses and ponies in North Somerset, England), the incidence of obesity was high (~28%) when animals were evaluated at the end of winter, although prevalence was even higher at the end of summer (~35.5%) (Giles et al., 2014). A more recent study, which looked specifically at ponies aged ≥ 7 years within 50 miles of the Royal Veterinary College, found 72% were overweight or obese (Menzies-Gow et al., 2017). Even animals which are being competed may often be overweight or obese. In a study of over 300 horses and ponies competing at a national unaffiliated championship, 41% were overweight (BCS > 5/9) and 21% were obese (BCS ≥ 7/9), with show and dressage horses being the most likely to be overweight (Harker et al., 2011).

In most cases, the reason for a horse being overweight is that they have stored excess energy/calories as fat, i.e., they have been overfed relative to their activity level. Many horses that spend most of their time in stables with occasional hacks may not require any more than maintenance energy intakes, and yet many are fed much more than this. Similarly, animals turned out to pasture at certain times of the year might be getting several times their energy/calorie requirement, especially as horses and ponies are kept more often on "improved" pastures, such as those established for dairy or beef production.

It is perhaps surprising that more animals do not become overweight, and this may reflect individual differences in activity levels when out in the field, as well as ability to convert feed to fat. Certain breeds (e.g., Quarter Horses and Morgans) and types (ponies) are perhaps more prone to obesity and benefit from even closer attention to their diet and exercise. However, many factors influence an individual's propensity for weight gain, including genetics, of course, but seasonality (Dugdale et al., 2011b) is perhaps an underestimated factor in that under feral conditions they tend to gain fat during summer months when food is abundant and then lose it during the winter. This natural pattern tends to be disturbed with domestication and the provision of rugs/stabling and feed throughout the winter months.

What has WALTHAM been doing over this time?

WALTHAM's journey into equine laminitis and obesity started with the collaborative research that we undertook initially with Dr. David Kronfeld and then Dr. Ray Geor and their students at the Virginia Tech MARE Centre in the late 1990s and early 2000s. Around the same time, we also started collaborating with Prof. Jonathan Elliott, and Drs. Simon Bailey and Nicola Menzies-Gow and their team at the Royal Veterinary College, London (RVC). In the mid-2000s, WALTHAM established the WALTHAM International Laminitis Research consortium followed by the WALTHAM International Obesity consortium. These consortia bring together world-leading equine veterinary, nutrition, and research experts interested in collaborating on the important topic of laminitis and obesity, resulting in many key publications in these areas.

With respect to obesity over the past 15 years or so, we have concentrated on four main areas:

1. How can we measure, define and monitor equine obesity? Example references: Carter et al. 2009; Dugdale et al., 2011a,b; Dugdale et al., 2012; Morrison et al., 2017a,b

2. What is the prevalence of equine obesity? Example references: Harker et al., 2011; Giles et al., 2014; Giles et al., 2015

3. Why does it occur and why is it important? Example references: Hoffman et al., 2003; Decker et al., 2007; Dugdale et al., 2011c; Giles et al., 2014; Giles et al., 2015a,b; Bamford et al., 2015

4. How is it best to manage the obese horse or an animal prone to obesity? Example references: Dugdale et al., 2010; Curtis et al., 2011; Longland et al., 2011; Argo et al., 2012; Tinworth, et al., 2010; Bruynsteen et al., 2014; Longland et al.; 2014, 2016; Bruynsteen et al., 2016

This work has led, for example, to deuterium oxide dilution becoming the gold standard method in horses to determine percent body fat (Dugdale et al., 2011b) and to the appreciation that horses and ponies with a body condition score of ≥7/9 should be considered obese (Dugdale et al., 2012). We have shown that breed differences exist in insulin sensitivity and insulin responses even in nonobese animals (Bamford et al., 2014) and those animals adapted to grain-based diets (Bamford et al., 2015). Perhaps most surprisingly, we have shown that obesity is not always associated with the development of insulin resistance and can, depending on the diet, result in an increase in insulin sensitivity (Bamford et al., 2015; Bamford et al., 2016a,b).

Pasture intake is a major factor for obesity (Giles et al., 2014), and we have observed that ponies can ingest up to 41% of their daily DM intake within a three-hour turnout period (Ince et al., 2011); up to 1% of body weight in DM within a three-hour period (Longland et al., 2016a), and nearly 5% of their body weight in DM per day (Dugdale et al., 2011c, Longland et al., 2011b). We have evaluated the use of grazing muzzles (Longland et al., 2016a,b), as well as types of haynets (Ellis et al., 2015a,b) as part of the management strategy for obese animals. We have shown that if required, and under veterinary supervision, marked dietary restriction to 1% of body mass in DM of appropriate, good-quality grass hay can be clinically safe (although significant ingestion of shavings can be a potential risk) (Curtis et al., 2011) and is likely to elicit weekly weight losses of ~0.5% of outset body mass weekly (Dugdale et al., 2010; Argo et al., 2012). Weight loss responses vary widely between individuals and, we have shown that some animals are more weight-loss resistant than others (Argo et al., 2012). In addition, the severity of any energy restriction may influence subsequent weight gain (Bruynsteen et al., 2016).

We have shown that whilst hay soaking can be useful it cannot be relied upon to reduce the level of water-soluble carbohydrate (WSC) to less than a specific content level, as the loss can be highly variable (Longland et al., 2011). The temperature of the water used to soak and the time of soaking will influence not only the loss of the WSC but also the microbial contamination as well as the extent of any dry matter loss (Longland et al., 2011; Longland et al., 2014; Moore-Colyer et al., 2014). This has resulted in the recommendations that hay composition analysis ideally should be undertaken post-soaking for laminitis-prone animals or a suitable hay or hay replacer chosen with a known NSC of ≤12 and preferably ≤10% on a DM basis. Importantly, we have confirmed that owners may be less able to correctly identify overweight animals by visual appearance alone (Potter et al., 2016; Morrison et al., 2017a,b) and we have, therefore, started to develop the body condition index as a potential additional monitoring aid (Potter et al., 2015).

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From the Beginning:

A Conversation with an Orthopedic Pioneer

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Can you give us a little insight into your background?

I was brought up in a small town in New Zealand, but I spent quite a lot of my school holidays at my aunt and uncle's high-country sheep station. They had cattle and sheep, and it's relatively remote. I thought the lifestyle was great, and when the vets came up, I thought it was a really interesting career. My aunt also rode and competed at show jumping. She taught me to ride, which transitioned me from looking at racehorses to actually riding horses, and I was hooked. I also spent time, as a high school student, in one of the local veterinary practices. I made the decision to pursue veterinary school at Massey University in New Zealand. I was interested, initially, in large-animal practice—sheep, cows, and horses. I actually worked in a mixed-animal practice for two years after I graduated, with a lot of surgery involved, because of sheepdog injuries. That was my start in orthopedic surgery. Sheepdogs get a lot of cruciate ruptures and bone fractures.

I left New Zealand in 1973 to lead a climbing expedition in the Peruvian Andes. After three months climbing and three months traveling in South America, I went to England and worked as a relief veterinarian in a large-animal practice in Wales for six weeks and a small-animal practice in the east end of London for about four months.

Then, after three months climbing in the European Alps, I started a one-year internship at the University of Guelph in Canada. At that time, surgeons were starting to save horses with colic. So the first thing that attracted me to equine surgery was the challenge of fixing horses with twisted bowels because many horses with colic were euthanized at that time. The internship involved all facets of equine surgery including orthopedics and lameness, and I became excited about all these pursuits. I decided, "This is what I want to do." So I applied for residencies and got a residency at Purdue University in Indiana, and by then I was certainly proceeding down the road to specialty surgery.

What drew you further into work with horses? And, ultimately, why did you view the horse as an ideal model for orthopedic research, specifically in surgery?

My internship at Guelph was in large-animal surgery, principally horses. I found that I enjoyed working around horses, and I worked well with horses. My initial goal when I went to Purdue was to be trained as a specialist in equine surgery. My advisor and mentor, Dr. Jack Fessler, gave me a research project in synovitis, inflammation of the lining of the joint, as part of my master's degree, which we did simultaneous with the residency program. This was a really critical juncture for me because two things happened.

First of all, I was working with an experimental model of synovitis and started to read the literature, which was virtually nonexistent in the horse. This was 1975 and 1976. The human literature on osteoarthritis was also quite confusing, being described as the arthritis just happening, with any inflammation secondary. What we showed in this study was that if you inflamed a joint and you did nothing else—if you didn't destabilize it or cause physical trauma—you could still get cartilage degradation. That was contrary to medical thinking in humans, and as I said, there wasn't much literature on the horse.

Then Dr. David Van Sickle asked me if I'd continue the work into a doctorate, in the same model, but looking at more outcome parameters and more questions. So I got very good training in joint disease because he had done so much research on the pathology of joints and established the Bone and Articulation Research Laboratory in the Purdue School of Veterinary Medicine. Much of his work was in canine joints, so I had the opportunity to learn a lot from him and to take it into the horse. And it was a big opportunity, because there'd been hardly anything done.

The second thing that was pivotal for my career was that I read about the arthroscope. The arthroscope was just beginning to be used as a diagnostic tool in humans. The state-of-the-art then was that if you had knee pain, you had an arthrotomy and your meniscus was taken out, based on the positioning of the pain. This was before MRI. Dr. Lanny Johnson, who was a professor at the medical school at Michigan State University, was having a course in diagnostic arthroscopy. And I guess I was cheeky enough to call him up and say, "I'd like to come to your course. I'm a veterinary surgery resident, not a human surgery resident." And he said, "Oh, it'd be great to have you. Come on up. I won't charge you a registration fee." So I drove up to Michigan State, and a couple hundred medical doctors and I learned how to do a diagnostic arthroscopy of the human knee.

So I went back to Purdue, and the university bought me an arthroscope so I could do diagnostic arthroscopy in analogous fashion to what they were doing in humans. I finished my doctorate degree, and I'd done a lot of arthroscopy, but just diagnostically. Then I got the job as an assistant professor at Colorado State University (CSU) in 1979.

That's when I started, with the help of a human orthopedic surgeon, Dr. Ron Grober, who visited me from Florida for a day, and got me started in developing triangulation techniques to do surgery. So, in other words, rather than just look, we were working to perform the surgical manipulation and visualization with the arthroscope. Those were the early days, when we were looking at the joint directly through the scope, and human orthopedists were doing it the same way. Those were early, pioneering days, and there was resistance to the technique both in human medicine and in the horse.

So I came to CSU equipped with a reasonable knowledge of joint disease. Plus, I had started using the arthroscope. Then we developed the surgical techniques. So my career has involved a research pathway and a clinical pathway. And, of course, they both join together.

Much of your clinical path has involved racehorse patients. How and why did you gain an affinity for racehorses and working with those patients?

Well, it was more a case of them getting affinity for me, because I had a technique that most other equine surgeons were not doing yet. I came here in August 1979 as one of four surgeons. Dr. Simon Turner soon got engaged in arthroscopic surgery here as well. By 1981, we had developed techniques to arthroscopically remove carpal chip fragments. We could also take chip fragments off the front of the fetlock joint arthroscopically. These were the two main surgical conditions in racehorses. So horses started coming here from 10 surrounding states. There were a couple of veterinarians doing some arthroscopy on the East Coast, but nobody else in the West. So we would get horses from Utah, Nevada, California, Nebraska, Kansas, Wyoming, Montana. There was strong racing in a number of those states at that time.

Starting in 1983, Dr. Turner and I started giving six arthroscopic surgery courses a year, and we could only take 12 people at a time because we're looking through the arthroscope. It was before we had video cameras, so it was very laborious.

Dr. Nancy Goodman, who was a CSU veterinary graduate, was in a racetrack practice in California, and she couldn't get into one of our courses because they were booked up. So she asked me to come down and do surgery on a couple of horses. I flew down to that clinic, and we operated on four horses and got done at 2:00 a.m. And then she had me back the following week for another four. And then I went for a weekend, and I ended up marrying Dr. Goodman. That started my surgical referral practice in Orange County, California. The first 16 years that Nancy and I were married, she worked eight months a year in California, and I was down there every other weekend doing surgery. When Nancy retired from racetrack practice after 20 years, in 2001, surgical practice continued with her as my primary assistant.

Fast-forward to now. A lot of horses that undergo arthroscopic surgery here at CSU are Quarter Horses in western performance disciplines. In the early days, we didn't have the techniques to treat stifle injuries, which are often seen in these equine athletes, such as cutting horses and reining horses. The stifle, which includes the femoropatellar and femorotibial joints, was the endgame because doing surgery on femorotibial joints, in particular, was more complicated. We developed a technique for femoropatellar joints and published it in 1986, but femorotibial joints came along after that.

Other techniques came pretty quickly with multiple techniques quickly developed by other equine arthroscopic experts in addition to our group. In the early 1980s, I certainly would not have predicted how far we would go with arthroscopic surgery and that we would be able to treat many racehorses for their injuries and have them come back to full athletic ability. Because of their multiple injuries, racehorses became the poster child for arthroscopic surgery. But it is now a powerful tool for treatment of joint injuries as well as problems of the tendon sheaths and bursae in all breeds.

A lot of people who get involved in racehorses are brought up with them. I wasn't, but I was always fascinated by racehorses. I used to bike up to the racetrack in my hometown of Oamaru when they had a meet. My mother didn't like it because that was gambling, and she was a good Presbyterian. But I was always fascinated by it. I got heavily involved in the racing industry, both racing Quarter Horses and racing Thoroughbreds, by virtue of operating on them. I love it, and I'm passionate about it. I still do surgery on them. For a long time, Nancy said the only way I liked horses was when they were on their backs with surgical drapes on them! I think she retracts that now and we currently have 12 horses at home. We did revolutionize things for racehorses with arthroscopic surgery in similar fashion to human orthopedics.

Arthroscopic surgery for equine athletes was the biggest revolution at the time in being able to treat musculoskeletal problems and get them back to their previous level of racing. While we developed a lot of the techniques for arthroscopic surgery, other equine surgeons did their share as well. We put on our first advanced arthroscopic surgery course at CSU in 1988, and we became the place where most veterinarians came to learn it. Our textbook *Diagnostic and Surgical Arthroscopy in the Horse*, whose fourth edition was published in 2015, has 454 pages reflecting the evolution.

You mentioned that you were fascinated by racehorses as a child and continue to be. Why? What about them have you found so captivating?

They are beautiful. And you see them with the jockeys dressed in their colors. It was fascinating, the whole thing—the speed and the excitement. Horse races were much better attended when I was growing up. In New Zealand, at that time, every race from across the country was on the radio on Saturdays. This was pre-television, as we got a black-and-white TV at home in my last year of high school. It was just like the days of Seabiscuit over here. Thousands of people went to the races; it was a real happening.

So let's fast-forward. Set the scene for us, in 1979, you're being interviewed to come to CSU. Whom did you interview with? What drew you to CSU? And what did you hope to accomplish here?

I interviewed here in 1979, and they had just opened the Veterinary Teaching Hospital on Drake Road. It had been open for two weeks. Dr. Jim Voss was head of the Department of Clinical Sciences. He had me stay at the Thunderbird Motel on the corner of College and Drake. The vet hospital was basically the only place on Drake that existed. Dr. Voss had a two-day interview process. You talk to everybody, and you give a seminar.

Then Dr. Voss was taking me to dinner with three other faculty members, including Dr. Simon Turner, who had been counseling me to shave off my beard, which I had at the time. So Dr. Voss picked me up in his pickup truck, and he was chewing tobacco, and I soon figured out I was in real cowboy country in the West. And he says, "OK, we've got 10 minutes for you to tell me what you think, what you like, what you don't like, and then we're going to get drunk." Well, it wasn't badly drunk, but we had a great dinner at the Prime Minister, and drinks certainly loosened things up.

During dinner, Dr. Voss asked, "Why did you shave off your beard?" He had been at a meeting where I'd spoken three months earlier. I said, "Well, Dr. Turner told me I couldn't communicate with you guys with hair on my face." He says, "Oh, that's no problem." And I said, "Well, I'll grow it back then." And Dr. Bob Shideler said, "Oh, it would be good if you didn't, Wayne." That's one of the two main things I remember about the interview. The other main memory was how much I wanted to get the job at CSU. I did get offered the job as an assistant professor, and I arrived in August 1979, still clean-shaven.

You're known at CSU as a University Distinguished Professor of Orthopaedics and as founding director of the Orthopaedic Research Center. But you've worn other hats through the years, including director of the undergraduate program in Equine Science. Tell us about those other roles.

That came about in 1994, when I'd been here 15 years. Dr. Bill Pickett started the Equine Sciences Program, which consisted of the undergraduate program in Equine Science and the Equine Reproduction Laboratory, and then he retired. Dr. Voss had become dean, and he was quite visionary. They had a search open for the new director of the Equine Sciences Program.

Dr. Voss called me and said, "I want to talk you into taking over Equine Sciences." That would mean taking over the undergraduate program and the Equine Reproduction Lab. But he also said, "I want you to build the biggest equine orthopedic research program there is." His plan was to replace me in the clinic and give me a tenure-track position in research as well. Nancy and I discussed it, and we decided it was a good opportunity if I was going to move forward. I wanted to continue surgery, and that was no problem because of my practice in Southern California.

For seven years, I was in charge of all three programs. I was building up the Orthopaedic Research Center and was director of the Equine Reproduction Laboratory, as well as the Equine Science undergraduate program. Then the Orthopaedic Research Center developed a critical mass, and I wanted to devote all my time to that. So that's how I had a seven-year swing through Equine Sciences.

You're an international pioneer in arthroscopic surgery and joint disease research in horses. And you've been honored many times by academic colleagues and others. As you survey your career, what do you consider your biggest achievements?

Pioneering arthroscopic surgery in the horse has been an achievement, along with teaching a lot of people how to do it, and writing the book on it *(Diagnostic and Surgical Arthroscopy in the Horse*,). And the second is developing the Orthopaedic Research Center. It started as the Equine Orthopaedic Center, but because of research grants from the National Institutes of Health and corporations, we've just left it as Orthopaedic Research Center. That's how we've got where we are now.

What do you consider to be your biggest, most important research breakthroughs or innovations?

They build on each other, as research does. Going back to my doctoral work, even though it's just one paper in the veterinary literature, recognizing the critical nature of synovitis actually turned out to be very important long-term for translational purposes. At the time, human doctors emphasized that osteoarthritis was not inflammatory, which seemed a bit strange, because it certainly was in the horse. Understanding that led into evaluating different treatments for the synovitis, and thereby making a lot of horses better. It has been important to validate the various treatments in joint disease as good, bad, or otherwise, and that is all part of the recognition of synovitis. Another big breakthrough was the gene therapy work that Dr. Dave Frisbie did with me for his doctorate, in collaboration with Dr. Christopher Evans, who was at the University of Pittsburgh and then moved to Harvard. We showed that interleukin-1 was the bad guy and that the equine interleukin-1 receptor antagonist gene, which is a natural antagonist, would shut down inflammation in the joint, and the consequent osteoarthritic change. This work was our first venture into the world of biologic therapies.

Our cartilage-healing research has been important, along with use of the horse as a model for cartilage repair in humans. Early diagnosis of musculoskeletal disease, because of the drastic consequences that you can have with catastrophic injury, is a huge part of our work. This started with Dr. Chris Kawcak's doctoral studies with Dr. Bob Norrdin and me showing how quickly microdamage could develop in the exercising horse and that this was the initial event in osteochondral fractures. While this microdamage could be displayed in pathology samples, we needed to be able to diagnose it before it became a critical fracture in the horse. We have made considerable progress in identifying imaging biomarkers, including nuclear scintigraphy, computed tomography, and MRI, as well as fluid biomarkers that we can pick up in the serum. This area is still a work in progress but has got the best potential of predicting catastrophic injury compared to other techniques.

The two biggest breakthroughs in sports medicine, whether it's horse or humans, are arguably arthroscopic surgery and biologic therapies. That's where we are now, as we transition into the Translational Medicine Institute. These are therapies that have minimal side-effects and take us to a newer level. They include proteins, cellular therapies, and stem-cell therapies. We have taken a problem that we treat arthroscopically, and we've been able to raise our success rates significantly with the additional use of bone marrow-derived mesenchymal stem cells.

Continuing this path of discussion, define translational medicine.

Transitional medicine is the use of basic laboratory research, preclinical research in vivo, and clinical examination that leads to patient success, with what we learn in animals often translating into improved medical treatment in humans. The outcome is better diagnosis and better treatment of the patient, whether animal or human.

What do you see as the role of biomedical research and veterinary medicine in the process you just described? Essentially, what is the unique contribution of veterinary medicine in that spectrum of discovery and improved care?

At the present time, you're never going to get a medication or a biologic technique validated and licensed for use in humans until you do good preclinical research in animals. So, pragmatically, you've got to have preclinical work conducted by veterinarians in animals before you can get it into humans. Additionally, many diseases that occur naturally in people also occur naturally in animals. That makes veterinary research and clinical treatment important in advancing human medicine: when we join our efforts and join our discoveries, we find more effective treatments more quickly.

Here, we're interested in musculoskeletal disease and injuries, such as osteoarthritis, cartilage injury, tendon injury. The horse gets these naturally, as does the person. With cancer, the dog is the translational starting point, because they develop so much cancer during the course of their lives, just as humans do.

Veterinary medicine is critical, and it's recognized a lot more, too. In the old days, it was like, "Well, animals are different than people." You'll still get some pedantic souls who talk like that, but there are lots of parallels.

Did you have an epiphany sometime during your education or your career, when you realized, "This work I'm doing could have far-reaching implications, not only for animal health, but for human health"?

It's been more of an evolution. I learned how to use an arthroscope from a human orthopedic surgeon. After that, we developed the techniques in equine arthroscopic surgery. We got into inflammation and recognizing the importance of synovitis through study in the horse. Now, there's lots of papers in human medicine on the critical nature of primary synovitis and primary subchondral bone disease, something that we've known ever since we started clinically treating horses and have also defined more closely with research. We've always felt that many findings in horses could be extrapolated to humans.

We started working with Dr. Richard Steadman at the Steadman Clinic and Dr. Bill Rodkey at the Steadman Philippon Research Institute in Vail because they wanted us to validate the use of microfracture as a surgical technique to repair damaged areas of articular cartilage of the knee. After that, we had corporations coming to us to test treatments in the horse, and later worked on quite a number of grants from the National Institutes of Health with us doing pivotal preclinical studies in the horse.

What do you think of as a best example of work you've done in the horse that has been applicable to human musculoskeletal disease?

We have worked collaboratively with experts in biomarkers in osteoarthritis. From that, we have developed biomarkers to predict early osteoarthritic change in the horse that also have a fairly good probability of predicting catastrophic fracture, or at least significant musculoskeletal injury, in the horse.

We worked with Dr. Chris Evans, who is arguably the father of gene therapy in human orthopedics, on the interleukin-1 receptor antagonist research. He pointed out that, for the first time, we showed with gene therapy we could get a clinical response close to a cure for osteoarthritis.

Our results with mesenchymal stem cells have been very impressive in the horse. The proof of principle has been accomplished. The optimal use of these cells given the current regulatory standards, including the need to ensure safety, is an evolving challenge. But we've been able to prove the value of these therapies and to stimulate further developmental efforts in human medicine. Our efforts are certainly a small part of the overall human landscape, and people such as Dr. Arnold Caplan at Case Western pioneered the work in bone-marrow-derived mesenchymal stem cells starting over 20 years ago. The advantage of the horse is that we've been able to do clinical studies and get good proof of principle of how they can significantly enhance our ability to treat osteoarthritis, cartilage disease, and tendon injury.

Can you provide a brief introduction to the Translational Medicine Institute?

The Translational Medicine Institute is an evolution from the Orthopaedic Research Center that we started in 1994 and built into a large research program (the largest orthopedic research center in a veterinary school anywhere in the world). The Translational Medicine Institute was a vision by Drs. Dave Frisbie, Chris Kawcak, and me that we sold to John and Leslie Malone. John agreed to be the lead donor. We had to get a matching donation and this was provided by Abigail Kawananakoa. We are going to continue what we have always done for horses but with a larger translational human component. In addition to a $77 million building, we have achieved partnerships with a number of critical programs in human regenerative therapies and sports medicine and one of the principal aims is to be able to not just develop therapies but fast-track them as much as possible into the human patient as well as the equine patient.

Looking forward, what do you see for yourself in the next several years?

I am in transitional retirement, as it is called at Colorado State University. Though I am trying to slow down a bit, it's not going very well at the moment. I have handed over administration to Drs. Frisbie and Kawcak and plan on retirement in another two years. Certainly, I will always have an office and be coming in, but I don't see myself losing any passion for what our program is doing. I want to stay involved with surgery and consultation in the equine industry and doing my bit to keep translating our vision into reality. I am leaving a large group of terrific people to carry on the cause. Other than that, my aim is to rock-climb more, spend more time with my wife, and visit my second home in New Zealand more often. ✦

Colorado State University supplied content for this article.

Advances in Neurology

Steven Reed, D.V.M., Diplomate A.C.V.I.M.

Can you tell us a little about your educational and professional background?

I graduated from The Ohio State University College of Veterinary Medicine in 1976. I served as an intern and then a resident in equine medicine and surgery at Michigan State University. At the end of the residency, I accepted a faculty position at the College of Veterinary Medicine at Washington State University. By 1983, though, I was back at Ohio State as an assistant professor, and by 1994 I was a professor. I stayed at Ohio State until I retired from academia in 2007 as an emeritus professor. I then moved to Lexington to work at Rood and Riddle Equine Hospital.

How did you become interested in neurology?

My interest in neurology started while in veterinary college, and the people who helped me develop this interest and help me grow as a diagnostician and veterinarian were Drs. Cheryl Chrisman, Joe Mayhew, Charlie Boles, and Barrie Grant.

In addition to these people, my career has been helped by many friends and colleagues, including Drs. Al Gabel, Larry Bramlage, Warwick Bayly, Debra Sellon, Martin Furr, David Granstrom, Dan Howe, and Yashuko Rikihisa. I know I am leaving off the names of others who were influential in my journey, but it is impossible to name all the people who helped me arrive where I am today.

Reaching back 30 years ago, what was the landscape like for horses with neurologic disease?

Back then, we certainly understood that horses had neurological problems, but they weren't always viewed as treatable. In fact, some individuals jokingly said neurology was a euphemism for necropsy. To be candid, at the time, some were and some weren't treatable, and there was not nearly as much hope for horses with neurological problems as there is today.

While our ability to make accurate diagnoses of neurological diseases has improved over the past 40 years, we performed many of the same diagnostic procedures as used today. For example, we performed myelograms, but used metrizamide as the contrast agent, and this had to be run through a Millipore filter as the final "sterilization" step before injecting it into the subarachnoid space. Today, we use ionic contrast agents such as lohexol, which comes to us as a sterile injectable formulation.

At that time we also began to realize that some of the bony lesions observed in the appendicular skeleton were similar to those seen in the axial skeleton, and the underlying cause was likely the same. We were beginning to learn about epigenetic factors, which were important in the development of these conditions, such as the role of dietary imbalances (particularly trace nutrients like copper and zinc), trauma, and gender (males three times more likely than females). We also noted that farms having OCD lesions in the stifle often had similar problems in the neck, so that made us think about timing of the developmental problems both in utero and post-foaling.

We were learning a lot about developmental orthopedic disease in young horses at this time, and we realized that the neck might be the most sensitive site for development of these problems. We also started to realize that there may well be treatments that, if initiated at the proper time, might still allow these horses to have an athletic career.

Aside from the problem of cervical vertebral stenotic myelopathy (CVM or wobbler syndrome), another very important neurological problem, equine protozoal myeloencephalitis (EPM), was starting to be recognized. Thirty years ago, little was known about EPM. Researchers were suspicious of a protozoal agent based on some early recognition by Dr. J.P. Dubey and later Dr. James Rooney, a pathologist who initially referred to this condition as segmental myelitis. After striking down various possibilities including toxoplasmosis, *Sarcocystis fayeri*, and some parasites, we finally recognize that *Sarcocystis neurona*, a protozoan parasite with the opossum as its definitive host, was the culprit.

In terms of wobbler syndrome, by 1979, there was a surgery being developed, though it didn't seem to catch on within the veterinary community or with horse owners.

Can you tell me a little about the time when researchers began looking at specific neurological problems in horses?

The pioneers and critical researchers looking at neurological diseases of horses included Drs. Sandy De La Hunta and Joe Mayhew at Cornell University and Drs. Barrie Grant and Pam Wagner at Washington State University.

Dr. J.P. Dubey is recognized as the most significant researcher of protozoal diseases in all species, not just the horse, and some of his colleagues, like Drs. Dave Granstrom, Dan Howe, and Clara Fenger led the investigation on equine protozoal myeloencephalitis (EPM).

When did you first begin to study or research neurology in particular?

I first started looking at wobblers as a resident. Though Michigan State was not a center for this research, Dr. Charlie Boles helped direct me to study things I was interested in and helped me decide to go to Washington State, where lots of work was being done on this problem.

In the 1970s, both CVM and EPM were coming to the front. As time went on, we learned about equine herpesvirus encephalopathy (EHP), equine degenerative myeloencephalopathy (EDM), and equine motor neuron disease (EMND).

Was there a disease that really got horsemen and veterinarians focused on neurology?

No doubt this was EPM because we finally had a treatable disease.

Fast forward 30 years, what does a neurological examination consist of today? How did your work in standardizing the neurological exam come about?

The goal of any neurological exam is to establish whether a neurological problem is present and to determine where exactly that problem is located. Once anatomical localization is found, additional testing must be employed to confirm the cause. That often includes radiography, myelography, collection and analysis of cerebrospinal fluid to include measurement of antibody concentrations against organisms like *S. neurona and N. hughesi*, or electrodiagnostic testing. These tests will help pinpoint the cause of the lesions.

Before examining a horse, I like to get a little background, if possible, including the horse's age, sex, breed, and use. While these aren't essential, they often help because horses of various breeds react differently or horses of certain ages may be more predisposed to certain conditions, for example.

I always begin an examination at the head and then proceed to the tail. Consistency is important because then there is less likelihood that I will omit any part of the examination. This craniocaudal approach can be used for both standing and recumbent horses. I generally follow the exam format laid out by Mayhew in *Large Animal Neurology: A Handbook for Veterinary Clinicians* (Lea & Febiger, 1989), which divides the exam into five components: head and mental status, gait and posture, neck and forelimbs, trunk and hindlimbs, and tail and anus. An equally useful text is *Equine Neurology* (Wiley Blackwell, M. Furr and S. Reed), published more recently in 2017.

Gait evaluation is another important aspect of the neurological exam, even from a young age. Common gait abnormalities in horses with neurologic disease include ataxia, spasticity, and weakness or paresis. Because postural reactions are sometimes difficult to interpret in horses, using gait abnormalities to help local a lesion is important. I nearly always place the feet and limbs in an unusual position to observe the response of the horse.

Evaluation of gait is critical because subtle neurologic gait deficits often go unrecognized or sometimes, incorrectly, may be considered insignificant. I observe the horse at the walk and trot, both on a straight line and on turns. I will ask some horses to negotiate a small obstacle such as a curb, to walk up and down a slight incline. If possible, I like to see the horse turned free. Elevation of the head and walking on a slope may exaggerate a subtle deficit and make it more noticeable.

The neurological grading system typically used is based on scores from 0 to 5: 0 (no gait deficits), 1 (barely perceptible deficits, worsened with head elevation), 2 (deficits noted at the walk), 3 (deficits noted at rest and at the walk, and nearly falls with head elevation), 4 (falls or nearly falls at normal gaits), and 5 (recumbent).

The neurologic examination has a degree of subjectivity built into it, but that subjectivity is waning given some of the technology that we have now, including the ease with which we can video these exams.

In terms of standardizing the neurological exam, I followed the leadership of Dr. Joe Mayhew and many of his disciples, such as Dr. Rob MacKay, and that led us to the aforementioned scoring system still in use today.

Beyond the neurological examination, we have come a long way in three decades. In terms of myelograms, which involves the injection of a contrast agent into the spinal canal to reveal compressive lesions, we now have more choices in contrast agents. Some of these are safer for horses compared to the ones we used years ago, as some horses had adverse reactions to some of the older radiopaque dyes, such as metrizamide.

Computed tomography (CT) and magnetic resonance imaging (MRI) scans can also help evaluate changes in bone structure, internal bleeding, inflammation, and other nervous system disorders such as neoplasms. CT scans are used primarily for injuries of the head and proximal spinal cord. MRI scans are especially helpful in cases of head trauma and help to pinpoint problem areas quickly.

Aside from the exponential leap in diagnostic procedures, what else has helped horses with neurological disorders?

From a nutritional standpoint, there is no question that oral supplementation of vitamin E has helped horses with equine degenerative myeloencephalopathy (EDM), a noncompressive, symmetric degenerative neuropathy in young horses characterized by weakness, ataxia, and spasticity. The earlier vitamin E treatment is initiated, the better the chance of response, it seems. Horses diagnosed with EDM should be supplemented with natural-source, water-soluble vitamin E, such as Nano-E from Kentucky Equine Research, as it's been proven to have greater bioavailability than synthetic forms and other natural forms.

Oral supplementation of vitamin E has been important in horses with equine motor neuron disease (EMND), a neurodegenerative disorder of the lower motor neurons of adult horses, characterized by neurologic and muscular dysfunction and muscle-wasting. EMND is an oxidative disorder, caused principally by a prolonged lack of dietary vitamin E. Prolonged vitamin E deficiency leads to chronic oxidative stress, resulting in death of motor neurons and myopathy.

What's on the horizon when it comes to neurology in horses?

I think there are two important things that we will see. The first involves genetic testing. I think in the near future we will have a better understanding of the role of genetics in many diseases. Already, for example, we know that wobbler syndrome and EDM has a genetic component. Perhaps there is a peculiarity in the genetic makeup that weakens a horse's immune system so it becomes susceptible to these problems.

Another example is EPM. In areas that have healthy populations of opossums that host *Sarcocystis neurona*, more than half of the horses in those areas will become infected with the protozoa. Less than 4%, though, will actually display neurological signs of the disease. Is there a genetic factor that makes these horses more vulnerable to the protozoa?

Second, there is promise in determining the genetic etiology of neurological diseases through the use of next generation sequencing platforms. Next generation sequencing has increased the speed at which DNA can be sequenced at a fraction of the cost of older sequencing technology. Certainly, advances are being made on this front to benefit human neurology, and I expect equine researchers to follow suit as soon as possible.

Further, I think veterinarians are going to rely more and more on CT, specifically portable units, which will be invaluable for use on extremities. This could be a game-changer for high-performance horses, like racehorses, three-day-event horses, and others that depend on soundness to do their job.

If you could be remembered for one thing, in terms of helping horses with neurologic disease, what would it be?

I'd like to think it would be something along the lines of this: it's OK for a horse to have a neurologic disease. Recognition of a neurologic problem doesn't necessarily mean that a horse's life or intended life will end or in many cases even change. Many of these conditions can be treated and managed, allowing the horse to have a useful and valuable life.

What work have other researchers done in this area that you feel will have a lasting impact?

The development of how to fix horses with CVM with better techniques by Drs. Barrie Grant, George Bagby, and others has a lasting impact, as has the diagnosis of EPM developed by Drs. Granstrom and Howe.

Are there any milestone events in the foreseeable future in terms of understanding neurological diseases in horses?

Dr. Carrie Finno at University of California, Davis, is nearing some breakthroughs with EDM, and she and her coworkers have used genetic testing to help get there.

Instead of an exhaustive reference list, can you suggest two or three review papers or book chapters that would provide an overview of the subject? Suggest something you've written or edited, if you like.

These two texts have a lot of great information in them presented by some outstanding veterinarians and researchers.

Equine Neurology, Second Edition. 2015. M. Furr and S. Reed, editors. Wiley Blackwell.

Equine Internal Medicine, Fourth Edition. 2018. S.M. Reed, W.M. Bayly, and D.C. Sellon, editors. Elsevier. ✦

Then and Now:

A Brief on Equine Muscle Disease

Stephanie Valberg, D.V.M., Ph.D. Diplomate A.C.V.I.M., A.C.V.S.M.R.

Q

Why did you choose to study muscle disease? What attracted you to this field of study?

I have been fascinated with horses since a very early age and have found my happy place training and competing in three-day eventing. Horses are supreme athletes whose beauty and performance depends upon their powerful musculature. Through years of careful genetic selection, equine breeders have shaped muscle form and function to produce breeds with muscles fine-tuned for extreme endurance, an elegant piaffe, a 1.5-meter jump, or a bold stretch across the finish line.

My doctorate concentrated on exercise physiology in horses and, while working on my degree, I was struck by the remarkable plasticity of muscle in response to conditioning and training. Through my veterinary education, it became clear to me that along with selection for positive traits, deleterious traits were also inadvertently incorporated into our horses. Even minor or intermittent perturbations in muscle function can have a major impact in horses because they are constantly being exercised and pushed towards maximal performance. It became my life's work to understand what muscle diseases were impacting the performance of horses and how we could best manage them.

As background, can you tell me a little about the time when researchers began looking at muscle problems in horses? Who were the pioneers in this field? Were there any landmark studies done in these early years that made other researchers take notice?

The most common muscle condition in horses, tying-up or exertional rhabdomyolysis (ER), is centuries old and well described in the literature of the 19th century. In 1883, the clinical signs of ER, or as it was then termed "azoturia," were described as "sweating and trembling, scarcely able to turn in the stall, the muscles of the back and loins in a state of spasm, tail quite stiff." Azoturia was known to develop in “animals rather handsome, well shaped, and good thrivers, than others differently constituted.” It occurred most often in the fall and winter in horses fed legumes or corn. Further, veterinarians had already recognized that “azoturia does not, or very rarely, attacks horses roaming at large in the fields, whether young or old; also that in all cases it is more apt to occur under conditions succeeding a period of rather smart or active work that followed enforced idleness.” The variety of terms used for this condition over centuries is as diverse as its proposed etiologies. Hysteria, lumbago, and black water were other early names for the condition.

In the early 20th century, elegant scientific studies by Birger Carlström definitively established that azoturia was a muscular disease with myoglobinuria arising from damaged muscle. Further, Carlström was able to reproduce ER in draft-type horses by feeding 3 kg of molasses during a period of rest prior to exercise. Muscle biopsy samples obtained during an acute episode of ER had muscle glycogen concentrations that were twice normal and muscle lactate concentrations up to three times normal. As a result of these well-designed studies, Carlström’s theory that a period of rest on a high-grain diet brought about a lactic acidosis that coagulated muscle tissue with exercise was pervasively held to be true for all breeds of horses for 60 years. Although lactic acidosis was later proven not to be associated with azoturia or tying-up, his observations were astute precursors to the discovery of polysaccharide storage myopathy (PSSM) in horses.

As lighter breeds of horses increased in popularity in the mid-20th century, confusion abounded as to whether the milder clinical signs of “tying-up” in lighter breeds of horses had a similar basis as azoturia in draft-type horses.

Meginnis, in 1957, further substantiated the view that tying-up and azoturia were different muscle disorders, noting from his practice that there were four significant differences between ER in light vs. draft breeds: (1) seasonality (none in racehorses); (2) history (fit racehorses worked daily unaffected by a change in ration); (3) handling (racehorses were less severely affected if walked during an attack); and (4) mortality (much lower in light breeds).

When did the upsurge in researching equine muscle problems occur?

From 1980 onward, there was a dramatic increase in the number of scientific and clinical investigations of ER in horses. Koterba and Carlson established that light breeds of horses with ER did not have a lactic acidosis but rather a hypochloremic metabolic alkalosis. Roneus and Lindholm determined that vitamin E and or selenium deficiency were not probable causes of ER in Standardbreds. The proposed theory of hypothyroidism causing ER was not substantiated by thyroid releasing hormone stimulation tests, T3 activity measured when horses were not receiving nonsteroidal anti-inflammatories, or by thyroidectomy.

Electrolyte disturbances, including intracellular potassium depletion, were proposed to cause ER. Substantial electrolyte imbalances were apparent in serum samples from endurance horses but not horses experiencing ER with light exercise. Fractional excretion of electrolytes measured in catheterized urine samples suggested that body stores of sodium or calcium were depleted in ER horse, and dietary supplementation was suggested to ameliorate episodes of ER. Subsequent studies, however, showed that there was marked variability in fractional excretion of electrolytes from day to day and horse to horse, and studies revealed no difference in total daily electrolyte excretion between Thoroughbreds with ER and healthy horses fed the same diet. A gradual recognition of the complexity of ER that affected a wide variety of equine breeds arose from these studies.

When did you first begin to study or research muscle disease? At that time, what were the primary research interests in the field? What was happening 30 years ago?

To delve deeper into the mystery of ER, researchers needed a thorough understanding of normal equine muscle responses to exercise; better diagnostic techniques to capture physiologic disturbances in ER muscle; a better understanding of muscle pathology; development of equine genome maps; and sequencing of the equine genome to characterize potential genetic defects.

During the latter part of the 20th century, an explosion of information and diagnostic techniques in human neuromuscular disorders would prove key to unraveling tying-up. Armed with these tools, we moved from theories of autointoxication or lactic acidosis to a more sophisticated understanding of multiple environmental and genetic causes of equine ER.

In those early years, what was considered state-of-the-art technology when it came to determining muscle disease? Was diagnosis based solely on clinical signs? What were those signs?

When I first started working on muscle disease, the only tools available were physical examination to determine if horses had firm or hard muscles, atrophied muscles, or alterations in the way they moved that would indicate a muscle disease. We also used blood samples to measure electrolyte concentrations and proteins, such as CK and AST, which are indicators of muscle damage.

In looking specifically at the work you've done, what has changed in the way you study muscle pathology? How has technology changed the way to diagnosis?

A window into the world of equine muscle opened when Arne Lindholm and David Snow, in the 1970s, adapted the percutaneous needle biopsy technique for use in horses. A modified 6-mm Bergrstrom needle made it possible to repeatedly sample unsedated horses and characterize the properties of equine muscle. I learned to use this technique during my doctoral work in Sweden and adapted it to use with horses that had muscle disease.

Neuromuscular diagnostic laboratories, such as the one I established at the University of Minnesota and now at Michigan State University, have formed the cornerstone for the identification of specific etiologies for ER in horses. A centralized site to receive muscle biopsies from across North America facilitates: (1) assimilation of the astute observations of large numbers of practitioners submitting biopsies; (2) accumulation of hundreds of cases from which to identify patterns of disease; (3) banking of biochemical and DNA samples that could be used to investigate the pathophysiology of disease subsets; and (4) formation of a research hub where breeders, equine practitioners, physiologists, biochemists, and molecular biologists could collaborate on muscle diseases in horses.

The remarkable development of genomic maps and the sequencing of the equine genome in the past 20 years provided new tools to investigate potential heritable bases for ER in horses.

Your accomplishments in the understanding of muscle pathology in horses cannot be undervalued. What do you feel are your greatest achievements?

- Convincing people that there was more than one muscle disease in horses and that tying-up was not caused by lactic acidosis;
- Introducing muscle biopsy as a diagnostic technique, which is now commonly practiced around the world;
- Identifying the genetic basis for overo lethal white syndrome (OLWS), glycogen branching enzyme deficiency (GBED), type 1 polysaccharide storage myopathy (PSSM1), and immune-mediated myositis/rhabdomyolysis; and
- Discovering that atypical myopathy in Europe was caused by ingestion of the toxin hypoglycin A found in sycamore seeds, making it possible to prevent this disease.

Can you tell us about the genetic work you've been doing in regard to muscle disease?

To date, we have identified the genetic basis for three muscle diseases: GBED, PSSM1, and a new mutation in MYH1. The MYH1 mutation is in a gene that codes for the fast twitch muscle's contractile protein myosin located in type 2X muscle fibers. About 8% of Quarter Horses have this mutation. When horses are exposed to certain environmental triggers (infection, muscle trauma), they develop either severe nonexertional rhabdomyolysis or atrophy associated with activating their immune system to destroy type 2X fibers.

What work have other researchers done in this area that you feel will have a lasting impact?

Dr. George Cardinet III at University of California, Davis introduced the muscle biopsy and histopathology to veterinary medicine. Dr. Gary Carlson had some seminal studies of tying-up. Dr. Jill Beech from the University of Pennsylvania New Bolton Center provided a solid foundation of research into tying up in horses. I worked with Dr. Jim

Mickelson from the University of Minnesota for many years who provided many valuable insights.

What have you done recently in this area? What can we expect from your research in the future?

We are working on diseases that are impacted by post translational modification of muscle proteins and how we can prevent detrimental modification of ion channels in muscle. We have an exciting project with Kentucky Equine Research looking at new antioxidants for horses.

How do you think other researchers will approach the study of muscle disease in the future?

I think new technologies and new applications in genomics will be very valuable.

What new muscle diseases have you uncovered lately? What technology was used to uncover them?

We are currently working on a disorder in Warmblood and Arabian horses called myofibrillar myopathy. We are using gene expression and proteomics to try to get to the bottom of what causes this disease.

Additional Reading Recommended by the Author

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Where We've Been and Where We're Going:

Understanding Protein Nutrition in Horses

Kristine Urschel, Ph.D.

Q

What attracted you to a career in research? Why did you choose to study protein requirements and metabolism?

While working on my undergraduate degree at the University of Alberta, I had the opportunity to do an independent study project with Dr. Ron Ball, an expert in protein and amino acid requirements in pigs. While that study ended up being a bit of a disaster, I loved doing research. Animal nutrition was my favorite undergraduate course, so I knew it was the area I wanted to pursue during graduate studies. As someone who grew up riding horses and participating in 4-H activities, I also knew that I wanted to eventually end up in the equine nutrition world.

While finishing my undergraduate degree, I began looking at graduate programs and found there were not any equine nutrition graduate programs available in Canada. At that time, I wasn't ready to leave Canada, so I accepted an offer to work on my doctorate with Dr. Ball at the University of Alberta. I made the decision that while working on my doctorate I would focus on how to do really great science, including learning a variety of research techniques that were not currently being used in horse nutrition research with the hope that, in the future, I could translate that knowledge and skill into the equine nutrition world.

My doctoral project consisted of using isotopes to study amino acid and protein metabolism in a neonatal piglet model for the human neonate. As I was finishing up my doctorate, a friend put me in contact with Dr. Ray Geor, an equine researcher at Virginia Tech. Although he was not doing protein research at the time, he was interested in this area and gave me the opportunity to do my postdoctoral training in equine nutrition, with the goal of adapting some of the techniques that I'd used during my doctorate for use in the horse. Also at Virginia Tech I had the opportunity to work with other researchers interested in protein and amino acid metabolism in other species and my interest in muscle protein metabolism developed.

As background, can you tell me a little about the time when researchers began looking at protein in horse diets? Who were the pioneers in this field? Were there any landmark studies done in these early years that made other researchers or nutritionists sit up and take notice?

Tough question! I researched all the way to the 1800s, but when I got there, I figured it was probably safe to make the statement that protein has been acknowledged as an important component of the equine diet, as well as the diets of humans and other animals, for well over 100 years. Dietary protein requirements were included in the first NRC publication *Recommended Nutrient Allowances for Horses*, which was published in 1949, and were discussed in several state experimental station bulletins well before that. Some of these early publications focused on the total amount of protein needed by horses at different life stages and the feeds that were good sources of protein.

In terms of "pioneering research," I'm going to focus on the research from 30 to 50 years ago, because that's when I think the research about protein nutrition in horses really started to take off. In the late 1960s, researchers at the University of Kentucky, particularly J.P. Baker, C.M. Reitnour, and colleagues, started to focus in on which segments of the gastrointestinal tract were important for protein digestion and found that the large intestine was a major site of protein digestion; however, in a later study they reported that the digestion happening prior to the large intestine had a much greater influence on blood amino acid levels than the digestion happening in the large intestine.

Around that same time, groups from both the University of California, Davis (L.M. Slade and colleagues) and Cornell University (H.F. Hintz and colleagues) looked at the ability of non-protein sources of nitrogen, in the form of urea, to be absorbed and retained by horses. Both groups found that horses were able to retain nitrogen originating from urea, but the effects were more pronounced when a lower quality protein was fed. Furthermore, Hintz's group reported that horses did not seem to be able to use the nitrogen coming from urea as efficiently as the nitrogen originating from amino acids in the feed ingredients.

Several years later, the group at Texas A&M (P.G. Gibbs, G.D. Potter, and colleagues) published a series of studies looking at the true digestibility of different forage and grain sources in different segments of the gastrointestinal tract. They found that while the small intestine was a major site for cereal grain and oilseed protein digestion, forage proteins were mainly digested in the large intestine. They concluded that the main absorbed end products from small intestinal protein digestion were amino acids, whereas the absorbed end product from large intestinal protein digestion was probably mainly ammonia. Collectively, these findings allowed researchers to propose that protein digestion in horses is more similar to nonruminant species, such as pigs and humans, than it is to ruminants, such as cows.

Another set of pioneering studies looked at the ability of different protein sources to meet the needs of horses, particularly growing horses. Research at Cornell University (H.F. Hintz and colleagues), University of Florida (E.A. Ott and colleagues), and Texas A&M (L.H. Breuer and colleagues) all identified lysine as a critical amino acid for the diets of growing horses. For all studies, when inadequate lysine was provided by the diet, horses showed lower rates of growth, and then growth increased when additional lysine was added to the diet. The group from the University of Florida also found that there were additional growth benefits when threonine, in addition to lysine, was added to the diet of growing horses. Similar to pigs and other nonruminant species, lysine is now widely regarded as the first limiting amino acid in typical horse diets, followed most likely by threonine.

These studies from 30 to 50 years ago set the stage for more recent research by providing critical information about the sites of protein digestion and absorption, and by identifying potentially limiting amino acids.

As a point of reference, how do horses compare to ruminants when it comes to protein digestion?

Anatomically, the gastrointestinal tract of horses is very different from that of ruminants, so the digestive processes are also quite different. The gastrointestinal tract of ruminants is arranged with the four-chambered rumen, the primary site of microbial processes, coming before the small intestine; in horses there is the stomach, followed by the small intestine, and then the large intestine, which is their major site of microbial processes (digestion of feed ingredients by bacteria and protozoa that live in the gastrointestinal tract). Microbes digest protein differently than the enzymes that the animal releases.

For nonruminant animals, such as horses, protein digestion starts in the stomach where acid and enzymes are released, continues in the small intestine using enzymes released from the pancreas and enzymes attached to the small intestinal wall, and then the amino acids are absorbed mainly in the small intestine. With microbial protein digestion, the microbes are able to completely digest protein, often right down to ammonia and carbon backbones. They then use these as building blocks to make their own amino acids, which they use to manufacture their own proteins. This has a couple of implications: (1) the profile of amino acids made by the microbes may be quite different from the profile of amino acids that was provided in the diet, and (2) much of this protein is contained within the microbe. In ruminants, these microbial processes happen earlier on in the digestive tract, prior to the sites of acid and enzyme release, so the microbes can be digested, and the protein can then be released and digested by enzymes to amino acids and absorbed in the small intestine. In horses, however, most of the microbial activity happens in the large intestine, which is after the main sites of protein digestion and absorption, so most of the microbial protein is probably not available to the horse. What we don't know is how well microbial protein made in other segments of the gastrointestinal tract can be digested and absorbed by horses.

When you first began studying or researching protein metabolism, what were the primary research interests in the field? When was this, and what was happening?

I've only been investigating protein in horses for about 11 years, beginning in mid-2007. After the burst of research in the 1960s through the early 1990s, there was a lull in equine protein research, as researchers focused on other areas of nutrition.

Interestingly, one of the more active areas at the time involved studying protein and amino acid metabolism in horses during different types of exercise. Several groups in the United States and Europe were looking at how blood amino acid levels changed in response to various types of exercise and how the supplementation of certain amino acids affected exercise performance, all in an effort to achieve a better understanding of how protein was being used during exercise and to gather clues for how diets could be modified to better support exercise in horses.

One of my all-time favorite horse studies was conducted around this time as well. The work was done by researchers in Japan (A. Matsui and colleagues) who exercised horses and then immediately intravenously infused either a saline, glucose, amino acid, or glucose + amino acid solution. Using stable isotope tracers, they were able to estimate rates of hind limb protein synthesis and degradation. They found the treatment most favorable to muscle growth included both amino acids and glucose after exercise, which is in line with what they were finding in exercising humans at that same time.

Other interesting research was being conducted by a group at Michigan State University (N.L. Trottier and colleagues), where they characterized the ability of different segments of the gastrointestinal tract to absorb amino acids. This group discovered that the genes for several of the amino acid transporters were found along the length of the small and large intestine and that lysine could be transported across this intestinal cell layer in both the jejunum and colon. They concluded that horses have the ability to absorb at least certain amino acids from the large intestine.

In the same vein, what was considered state-of-the-art technology when you began your work with muscle?

My interest in doing muscle research also started in about 2007, although I had been following research progress related to this area in humans and pigs for several years before that at scientific meetings. The study that I referred to previously, with the exercising horses in Japan, was, and to a large extent still is, state-of-the-art for equine research. In humans and pigs, state-of-the-art techniques being used were stable isotope methods to estimate rates of muscle protein synthesis and Western blot analysis to study the signaling pathways regulating protein synthesis. For the stable isotope methods, a stable (i.e., nonradioactive, nontoxic) isotope of an amino acid, generally either phenylalanine or leucine, is infused intravenously into the individual and then muscle samples are obtained at various time intervals. The theory is that this labelled amino acid will be incorporated into protein just like the nonlabeled amino acid, but because you will be able to track this labeled amino acid, you can then calculate the rate at which it is incorporated into the muscle protein and the rate of muscle protein synthesis. Western blot methods allow you to measure the abundance and in some cases the degree of activation of specific proteins of interest in muscle samples. This is done by first using gel electrophoresis techniques to separate the muscle proteins by size and then incubating a membrane containing these separated proteins with an antibody specific to the protein of interest. The membrane is then incubated with antibody that will recognize the original antibody and generate a light signal that can be captured either on x-ray film or by certain pieces of laboratory equipment. The more protein of interest that is present in the sample, the more of the antibody that will bind to the membrane and the more intense that generated signal will be.

In the 2000s, interest in the mTOR signaling pathway, the pathway that is now widely accepted as the one that regulates protein synthesis, really took off. So, at this time, there was a lot of interest in looking at how various anabolic stimuli such as feeding, amino acids, hormones, and exercise activated the different specific proteins in the mTOR pathway, in various physiological states.

Specifically, what areas related to protein nutrition and metabolism have you focused on in your research program? What technology has helped you since you began your work?

Since I started working in equine protein nutrition and metabolism, my research program has had two key areas of focus: (1) understanding the factors that regulate muscle mass in horses across the lifespan, and (2) assessing dietary protein and amino acid adequacy across the lifespan.

For my muscle research, I've relied heavily on literature relating to mTOR signaling in humans and pigs, and have used primarily Western blot techniques. Because we know so much more about how these pathways are regulated in these other species, it gives me a good idea of potential targets of interest to look at in horses. Furthermore, over the last several years, there has been a shift from looking at protein synthesis (i.e., mTOR signaling) to looking at markers for protein degradation as well. Because it is the balance between muscle protein synthesis and muscle protein degradation that will ultimately determine any changes in muscle mass, knowing what proteins may be good markers for muscle protein degradation, gives me a great starting point as I look to understand these pathways in horse muscle. One technique not yet optimized in horses is the isotope infusion method that will allow us to measure rates of muscle protein synthesis in the muscle. Getting that method up and running would allow us to see how mTOR signaling pathway activation translates into changes in the rates of muscle protein synthesis that occur.

For my second research area, I have used stable isotope infusion techniques to estimate whole-body rates of protein synthesis and amino acid requirements. These are the methods that I was referring to in my answer to the first question, that I began adapting for use in the horse while I was completing my postdoctoral training at Virginia Tech. To estimate whole-body rates of protein synthesis, a stable isotope of an essential amino acid, in our case [1-13C]phenylalanine, is infused intravenously or given orally at a continuous rate. Blood and breath samples are collected frequently, and we measure the amount of 13C-phenylalanine in the blood (as a percent of total phenylalanine) and the amount of 13CO2 in the exhaled breath. Using these values, we are then able to calculate how much phenylalanine is moving in and out of the free phenylalanine pool over a period of time, which is termed phenylalanine flux. We can also calculate the rate at which phenylalanine is being converted to carbon dioxide. Because the two major metabolic fates of essential amino acids are incorporation into protein (protein synthesis) and metabolism to carbon dioxide, if we know the flux and the rate of conversion to carbon dioxide, we can estimate phenylalanine use for protein synthesis by taking the difference between these two values, which provides us with an estimate of whole-body rates of protein synthesis. The same sort of isotope infusion procedure can be used to estimate protein and amino acid requirements using the indicator amino acid oxidation method (IAAO).

With IAAO studies, subjects will be studied while receiving graded levels of intake of the amino acid of interest (i.e., lysine), usually at least six different levels of intake. The isotope infusion methods described above will be used for each treatment, and blood and breath samples will be collected. With this method, we are interested in how the oxidation of phenylalanine to carbon dioxide changes with increasing levels of test amino acid intake (assuming the intake of all other amino acids is held constant). The theory is that when the test amino acid (i.e., lysine) is limiting to protein synthesis, none of the other amino acids, including phenylalanine, will be optimally used for protein synthesis and these amino acids will need to be metabolized to carbon dioxide. As test amino acid intake increases towards requirement, more and more protein synthesis will occur, so fewer of the other amino acids will be in excess and will need to be oxidized. The requirement for the test amino acid is the intake at which phenylalanine oxidation to carbon dioxide is minimized and stays at plateau levels (i.e., additional intake of lysine does not result in any further reductions in phenylalanine oxidation).

The IAAO method is considered to be one of the gold standard techniques for determining amino acid requirements in humans, according to the World Health Organization. We have used the phenylalanine isotope infusion methods in horses to determine whole-body rates of protein synthesis and to attempt to estimate amino acid requirements using the IAAO technique.

How has the work that you and your research team accomplished in this area furthered the understanding of protein metabolism?

In the last 10 years, my research team has been able to study protein metabolism in horses of all ages, from six months old to more than 30 years of age. In some cases, we have used muscle biopsy and Western blot techniques to look more specifically at muscle protein metabolism, and in others we have used the isotope methods to study whole-body protein metabolism.

For our work looking at the regulation of muscle protein synthesis and degradation in horses, we validated that commercially available antibodies designed against the human proteins would cross-react with the equine forms of the protein, confirmed that the activation of the protein synthesis signaling pathway was not affected by the depth of the gluteal muscle where the sample was collected, and determined that biopsies could be taken from the same muscle for five consecutive days with no effects on the activation of the signaling pathway, as long as a nonsteroidal anti-inflammatory was administered.

Once the methods were developed, we then conducted studies to determine how the activation of these signaling pathways was affected throughout the lifespan. In one study, we determined that the activation of muscle protein synthesis in response to feeding stimulus was more pronounced in younger growing horses (yearlings) than in mature horses and that this increased sensitivity to anabolic stimuli declined throughout the growth period. One of the main implications of this research is that it demonstrates that the window of opportunity to have the most effect on muscle growth is when the horse is young and if there are problems (nutrition, illness, injury, etc.) that occur during this phase that limit muscle growth, it may be more difficult to build that muscle later in life.

We also investigated these signaling pathways in old horses (>20 years) in comparison to mature horses, and between two different groups of horses, those with and without pars pituitary intermedia dysfunction (PPID). We found that the older horses did have lower activation of some factors in the protein synthesis signaling pathway, compared to mature horses. However, when we compared healthy old horses to those diagnosed with PPID, we did not see differences in the activation of any of factors studied in response to feeding stimulus. Furthermore, contrary to popular belief, we did not find any evidence to support that old horses with PPID were less sensitive to insulin than presumably healthy old horses of the same age, at both the whole-body and muscle levels.

Another study using old horses found that when they were treated with a nonsteroidal anti-inflammatory drug for four weeks, there was an increase in whole-body rates of protein synthesis, suggesting that the previously described low-grade chronic inflammation in horses may be impacting body protein metabolism. Together, the results from the old-horse study provide insight into the loss of muscle mass that occurs in horses (and other species) with advancing age. A significant take-home message from this area of my research program is that horses seem to respond similarly to anabolic stimuli as other species, so research from other species should provide us insight into how we can optimally develop and maintain muscle mass in horses.

In our first study that we conducted looking at dietary amino acid adequacy, we used the isotopic methods to investigate whether reducing dietary crude protein intakes from levels that are typical when commercial feeds are fed in combination with a forage source (~30% above requirement) to a level that just meets the current NRC requirements would still support similar rates of whole-body protein synthesis in weanling horses. Whole-body protein synthesis was lower when weanlings received the reduced protein diet, despite the fact that this diet still met the current NRC requirements. The results from this study indicated that the reduced protein diet was limiting in one or more of the indispensable amino acids and underlined the importance of determining the requirements for the individual indispensable amino acids.

As a follow-up to this initial study, we studied whether threonine was a limiting amino acid when weanlings were fed a commercial concentrate and a grass hay forage. In this study, we found that as long as a high-quality grass hay was fed, threonine did not appear to be a limiting amino acid. However, based on a high degree of variation in the threonine contents between different batches of grass hay that we have used over the past several years, this is something that needs to be investigated with a lower quality forage. We have also attempted to determine lysine requirements in yearlings and threonine requirements in yearlings and mature horses using the IAAO methodology. In all cases, the lowest level of test amino acid intake (either lysine or threonine) was still greater than the actual requirement, and we were unable to define an exact requirement. We also found that in old horses (~20 years old) receiving timothy hay cubes and a commercial concentrate, that there was no effect on whole-body protein synthesis when either lysine and threonine or lysine, threonine, and methionine where added to the diet. Interestingly, regardless of what amino acids were added to the diet, all of the horses were in negative nitrogen balance, indicating that they were losing body protein. In all of our studies to date, we have used a good-quality grass forage and typical feed ingredients. So, one of our major findings is that when a good-quality forage is fed and typical concentrates are used to formulate a diet that meets both the crude protein and digestible energy requirements, it is difficult to create a diet that is actually limiting in an essential amino acid. Because good-quality forage is not always available to horse owners, it will be important in the future to determine the requirements for the essential amino acids, particularly lysine and threonine, when a lower quality forage is fed.

What are you currently working on? What are the goals of the current work?

I have a few active areas of research relating to equine protein nutrition and metabolism. First, I have a doctorate student who recently defended her dissertation, which focused on how protein metabolism in horses is influenced by insulin dysregulation. She mainly used an induced model of insulin dysregulation but also did one study in horses diagnosed with equine metabolic syndrome. She has several manuscripts in the pipeline, but her major take-home finding was that protein metabolism is different in horses with insulin dysregulation, compared to healthy horses. From a signaling pathway standpoint, protein balance in horses with insulin dysregulation seems to be tipped more in the direction of protein breakdown (rather than protein synthesis), but much work still needs to be done to determine what the implications of this are and whether these differences in protein metabolism can be mediated.

I have a master's student who is examining whole-body protein metabolism in growing and mature horses consuming primarily forage-based diets and determining whether or not there any limiting acids. In mature horses, she found that both timothy and alfalfa hay cube diets seem to be able to meet all the amino acid needs for horses at maintenance; however, the alfalfa hay cubes provided much more protein than the horses could use, resulting in a lot of excess amino acids that the body needed to metabolize and excrete. In yearling horses receiving a mainly forage diet (~75% forage), there did seem to be benefits to feeding an alfalfa hay rather than timothy hay, in terms of being able to support greater rates of whole-body protein synthesis. This study in growing horses did not set out to identify what amino acids may be limiting in the predominantly timothy hay diet, but that is certainly a next step that we would like to pursue.

The last current study is probably the one that I'm the most excited about because it is one that takes me into the area of exercising horse protein metabolism for the first time. We are doing this study in conjunction with Kentucky Equine Research with the goal of looking at how feeding in proximity to exercise can influence whole-body protein metabolism and muscle mTOR signaling. We are in the early stages of this study, so no results to share yet, but I'm so excited to see what we find and how it compares to what has been reported in human athletes.

During your career, what other areas of study have you become interested in? Have you been able to pursue those?

There are so many areas that I have become interested in over the last 10 years that I would love to pursue with regards to protein nutrition and metabolism research in horses! Some of these areas include developing isotope methods to directly measure rates of muscle protein synthesis; studying the muscle signaling pathways in other physiological states such as lactation and continuing to look at how these pathways are influenced by metabolic conditions, old age, and exercise; and investigating how microbes in the gastrointestinal tract may contribute to whole body protein metabolism. Truthfully, for all of these, the greatest barrier to pursuing these areas of interest is the general lack of available grant funds to conduct equine nutrition and metabolism research.

What else can we add that might be interesting to horse owners and veterinarians?

The one thing that I haven't mentioned elsewhere is that there are a lot of supplements on the market that include amino acids or advertised as muscle-builders. Some of the common amino acid supplements include tryptophan and the branched-chain amino acids (BCAA), such as leucine, isoleucine, and valine. For these types of supplements, there is definitely a scientific basis for why they are being marketed but not necessarily the scientific data to back up the claims, particularly at the doses provided by typical supplements for horses. So, in these cases, buyer beware!

Tryptophan is sold as a calming agent, because it is a precursor for the neurotransmitter serotonin, which regulates processes such as mood and sleepiness. However, to the best of my knowledge, there are no studies actually showing that tryptophan, at the doses provided in commercial supplements, actually modifies mood or behavior in horses.

BCAA supplements are marketed for a couple of reasons, primarily to reduce fatigue and to promote muscle growth. In terms of reducing fatigue, the rationale is that they compete with tryptophan for transport across the blood-brain barrier and, if there are more circulating BCAA, there will be less tryptophan that moves into the brain for conversion to serotonin. BCAA are also the only amino acids that can be completely metabolized within the muscle, so they can act as a muscle energy source, sparing glucose and potentially reducing lactic acid formation. Unfortunately, in the few studies that have been done, there is no evidence that it improves performance or reduces the onset of fatigue, although at low intensities of exercise BCAA supplementation did reduce plasma lactate concentrations.

The use of BCAA to promote muscle protein accretion is supported by the fact that in other species leucine has been shown to be a potent activator of mTOR signaling. Unfortunately, at this time, there have been no studies examining the amount of leucine necessary to achieve this same effect in horses.

Finally, in the last few years there have been several feeds and supplements released claiming to promote muscle growth, without necessarily involving exercise. From what we know in other species, unless the horse was previously protein malnourished or is a growing animal (where they are in a stage of development where they are programmed to build muscle), feeding protein alone is not enough to promote the gain of muscle in a mature, sedentary animal. In the same way that humans cannot sit on the couch and eat steak all day and expect to look like a bodybuilder, mature horses cannot gain muscle without a combination of good-quality dietary protein and appropriate stimulation of the muscles through exercise. There are still a lot of opportunities for research when it comes to developing effective protein and amino acid supplements for horses!

Additional Reading Recommended by the Author

Surprisingly, there have been few review papers looking at protein nutrition in horses, and the ones that do exist are not recent. So, my first recommendation is a shameless plug, but it's a chapter on protein nutrition in horses that Dr. Laurie Lawrence and I wrote several years back:

Urschel, K.L., and Lawrence, L.M. 2013. Amino acids and protein. In: R.J. Geor, P.A. Harris, and M. Coenen, editors, Equine applied and clinical nutrition. Saunders Elsevier, St. Louis, MO, p. 113–135.

My second recommendation is actually a human review paper, but I've decided to include it because it provides a thorough overview of the factors that regulate muscle mass and how modifications in diet and exercise contribute to this regulation. Although very little of this work has been done in horses, I think it gives us some ideas for things that could be incorporated into our management strategies to optimize or maximize muscle growth.

Witard, O.C., S.L. Wardle, L.S. Macnaughton, A.B. Hodgson, and K.D. Tipton. 2016. Protein considerations for optimising skeletal muscle mass in healthy young and older adults. Nutrients 8: 181. doi: 10.3390/nu8040181

There's so many original research papers that I think have made important contributions to the area of equine protein nutrition that it's too hard to pick any of them to single out. Instead, if you want to learn more about any of the specific areas of equine protein nutrition and metabolism, please contact me and I would be happy to provide you with some suggestions for papers to look up. ✦

Vitamin E Nutrition in Healthy and Neurologic Horses

Stephanie Valberg, D.V.M., Ph.D., Diplomate A.C.V.I.M., A.C.V.S.M.R. and Carrie Finno D.V.M., Ph.D.

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Can you provide a little background about vitamin E?

Vitamin E was discovered in 1922 as an element essential for normal reproductive function in rats. Over the next 50 years, evidence emerged indicating vitamin E was also important for normal function of the immune, neurologic, and muscular systems. In horses, vitamin E research has mainly revolved around the role of vitamin E in maintaining normal function of the immune and reproductive systems, in optimizing athletic performance, and in studying diseases associated with vitamin E deficiencies.

Vitamin E describes a closely related family of eight fat-soluble naturally occurring compounds. Alpha-tocopherol is the most potent antioxidant of these eight compounds and therefore the most widely studied across species. When assessing "vitamin E" concentrations in horses, alpha-tocopherol is the isoform that is actually measured.

Expand on the work done with vitamin E on immunity and exercise in horses.

Regarding immune function, the high polyunsaturated fatty acid content of immune cells puts them at especially high risk for oxidative damage. In many species, studies have shown that lymphocytes are impaired by alpha-tocopherol deficiency. Further, alpha-tocopherol supplementation in rats repairs some of the age-related changes in the immune system.

In horses, the role of alpha-tocopherol in the humoral immune response was demonstrated by vaccination against tetanus toxoid and equine influenza virus in 15 horses in the mid-1980s. An increased immunoglobulin G (IgG) response was demonstrated in horses that received either vitamin E or vitamin E and selenium. Horses supplemented with synthetic alpha-tocopherol demonstrated increased bacterial killing capacity of monocytes and neutrophils. These studies were the primary justification behind the National Research Council's decision to increase the recommended dietary level of alpha-tocopherol from 15 IU/kg DMI (maintenance and growth) in 1978 to 50 IU/kg DMI and 80 IU/kg DMI (maintenance and growth, respectively) in 1989 as part of *Nutrient Requirements of Horses*, Fifth Edition.

Regarding vitamins and exercise, early studies in horses showed no apparent increase in measures of oxidative stress with exercise; however, more recent studies have demonstrated that oxidative stress is elicited with intense exercise in horses. Alpha-tocopherol supplementation in exercising horses has been studied with mixed results. Some studies demonstrate no effect on exercise-induced oxidative stress. In response to intense exercise, elite three-day-event horses at the two- and three-star levels showed no change in plasma alpha-tocopherol concentrations. Additional research is needed to determine if there are any detrimental effects of high antioxidant supplementation in exercising horses.

What are the current equine dietary recommendations for vitamin E?

The 2007 National Research Council (NRC) recommendations, as outlined in *Nutrient Requirements for Horses*, Sixth Edition, for dietary vitamin E requirements in horses are provided in IU/kg feed based upon an assumed total daily dietary forage intake of 2–2.5% of the horse's body weight. For adult horses, the recommended dietary amount ranges from 1–2 IU/kg/day, amounting to 500–1,000 IU/day for a 500-kg horse.

Horses obtain the highest amount of vitamin E from grazing pasture, ranging from 350–3,000 IU of vitamin E/day when consuming 1.7% of their body weight on a 90% dry matter diet of pasture. This range is variable because the vitamin E available on pasture is subject to change based on the time of year.

Vitamin E concentrations degrade when feedstuffs are processed and stored. In one study, a significant decrease was noted between the first and fifth cutting of alfalfa hay, and another study demonstrated that storage losses can reach 50% in one month. As evidence, a seasonal variation in plasma vitamin E concentrations was found with increased plasma vitamin E in the summer when horses grazed on fresh pasture and were fed fresh hay compared to the winter when horses were fed dried hay and oats.

One of the challenges with nutritional recommendations is that older research papers, book chapters, and reference materials often do not specify the formulation of vitamin E they are discussing. The actual formulation has a huge impact on the bioavailability of vitamin E. As discussed previously, vitamin E is a general term used to describe a family of eight different fat-soluble naturally occurring closely related compounds.

The family consists of two subgroups: tocopherols (saturated) and tocotrienols (unsaturated). Within each subgroup, there are four individual isoforms (alpha, beta, gamma, and delta). Alpha-tocopherol, specifically the RRR stereoisomer, is the most bioavailable isoform due to preferential uptake by the liver. For this reason, many equine supplements now strongly market the "natural" (i.e., RRR) form of alpha-tocopheryl available as an acetate (powder or pellet) or the most highly bioavailable form, alpha- tocopherol water-soluble (liquid) formulation. Many feeds, however, use synthetic vitamin E, all-rac-alpha-tocopheryl acetate, which has relatively low bioavailability. Thus, it is really difficult to know what the vitamin E requirements are if the specific formulation of vitamin E is not taken into account.

Can you mention and describe the equine diseases associated with vitamin E deficiency?

Vitamin E deficiency can exist in horses that have no outward sign of disease. A number of factors acting together, including the age of the horse when deficient, other oxidant stressors, the duration of vitamin E deficiency, and genetics, all probably determine whether clinical signs of disease will be evident. There are three specific disease conditions consistently associated with alpha-tocopherol deficiency: neuroaxonal dystrophy/equine degenerative myeloencephalopathy, equine motor neuron disease, and vitamin E deficient myopathy.

Equine Neuroaxonal Dystrophy/Degenerative Myeloencephalopathy

When young horses with an underlying genetic predisposition for equine neuroaxonal dystrophy/degenerative myeloencephalopathy (eNAD/EDM) experience vitamin E deficiency, they can develop signs of ataxia that closely resemble wobbler syndrome. The abnormality in these cases, however, lies in specific tracts in the nervous system unrelated to compression of the spinal cord. Equine neuroaxonal dystrophy (eNAD) describes a morphologic abnormality of select neurons and their axonal processes in the nervous system, whereas EDM appears to be a more severe pathologic expression of eNAD. Clinically, eNAD and EDM are indistinguishable. Histologic lesions in both eNAD and EDM consist of dystrophic neurons and axons, vacuolization, and spheroid formation, with the only difference being the distribution of the lesions. As these two terms most likely represent a spectrum of the same disease, eNAD/EDM is used to describe the overall phenotype.

Clinical cases of eNAD/EDM have been reported in several breeds, including Standardbreds, Paso Finos, Quarter Horses, Mongolian Horses, Appaloosas, Haflingers, Arabians, Morgans, Lusitanos, Thoroughbreds, Paints, Tennessee Walking Horses, Norwegian Fjords, and various mixed breeds. There is no sex predilection, and age of onset ranges from birth to 36 months, although most cases demonstrate clinical signs by six to 12 months of age.

There is strong evidence of a genetic component in that many case-report clusters involve related horses. In a family of Appaloosas, an unaffected mare produced four affected foals, one of which went on to sire seven foals, five of which were affected. One of these five affected foals sired nine offspring, eight of which developed signs of ataxia before one year of age. A prospective breeding trial performed in Morgan horses revealed a significantly higher number of affected animals when affected horses were mated as compared to control breedings. Mode of inheritance appears to be autosomal dominant with variable expression or polygenic. A study of risk factors associated with the development of EDM found that foals from dams that had an EDM-affected foal were at a significantly higher risk (25 times more likely) of developing EDM than foals from other dams.

Clinical signs in all cases include symmetric ataxia that is often more severe in the pelvic limbs than the thoracic limbs, abnormal base-wide stance at rest, and conscious proprioceptive deficits. In some reports, hyporeflexia of the cervicofacial and cutaneous trunci is described in addition to an absent laryngeal adductor reflex. Horses with eNAD/EDM that survive to two or three years of age commonly exhibit lifelong, stable neurologic deficits.

The developing nervous system is dependent on adequate vitamin E for normal development, and vitamin E appears to play a role in the pathophysiology of eNAD/EDM. Although vitamin E deficiency has been reported in some cases of eNAD/EDM, low alpha-tocopherol levels are not present consistently in all cases. Serum alpha-tocopherol concentrations were not significantly different between EDM-affected horses and control horses in many studies. However, recent work has demonstrated that there is a significant difference in both serum and cerebrospinal fluid (CSF) alpha-tocopherol during the first six months of life in eNAD/EDM affected foals. As older studies examined horses older than six months of age, they most likely missed the "risk period" for vitamin E deficiency, which occurs during early post-natal life.

Vitamin E supplementation of susceptible horses (i.e., on the same farm as previously diagnosed horses) appears to lower the severity and overall incidence of eNAD/EDM. Foals from an EDM-affected stallion compared to controls from an unaffected stallion had significantly lower plasma alpha-tocopherol concentrations, and it was concluded that vitamin E is a factor in the development of EDM in the first year of life in genetically predisposed foals. Foals with EDM do not demonstrate significant differences in oral vitamin E absorption as compared to controls. Overall, there is strong evidence that eNAD/EDM is an inherited disorder, and it may be that specific genetic polymorphisms in genes involved in vitamin E metabolism determine individual susceptibilities to eNAD/EDM under the same conditions of vitamin E deficiency.

Antemortem diagnosis of eNAD/EDM is based solely upon clinical signs, the elimination of other causes of neurologic disease, and a possible association with a low serum alpha-tocopherol concentration. At this time, a definitive diagnosis is only available upon histopathologic evaluation of spinal cord and brainstem tissue at postmortem. There is no treatment for eNAD/EDM, and there have been no reports of spontaneous resolution. Suspected cases are often treated empirically with vitamin E supplementation due to the association of low serum vitamin E concentrations with the disease. However, neurologic deficits do not improve with supplementation once the horse is more than six months of age.

Although the neurologic abnormalities appear to stabilize by two to three years of age, these horses are neurologically abnormal and often unfit for any performance activity. Prevention of the disease has been reported in genetically-susceptible herds by supplementing susceptible animals with 2,500-5,000 IU/450 kg/day water-dispersible natural (RRR-) alpha-tocopherol. Recent evidence supports supplementation is not necessarily entirely effective in preventing eNAD/EDM, but vitamin

E supplementation can decrease the severity of disease in foals born in a susceptible herd with previously diagnosed eNAD/EDM cases.

Equine Motor Neuron Disease

Equine motor neuron disease (EMND) is another neurodegenerative disorder related to a deficiency of vitamin E in adult horses. It affects a different part of the nervous system than eNAD/EDM, namely the lower motor neurons in the ventral horns of the spinal cord and selected brainstem nuclei. Clinical signs of EMND include weight loss due to muscle wasting, muscle fasciculations, and prolonged recumbency.

A definitive diagnosis of EMND is based upon postmortem demonstration of degeneration and loss of motor neurons from the ventral horns of the spinal cord. Antemortem diagnosis of EMND is based upon either the biopsy of the ventral branch of the spinal accessory nerve or the finding of neurogenic atrophy of predominantly type I muscle fibers on a muscle biopsy of the sacrocaudalis dorsalis medialis muscle, where type I fibers are found in sufficient quantities to evaluate. Examination of this muscle biopsy has a sensitivity of approximately 90%.

EMND appears to be an oxidative disorder, as dietary deficiency of vitamin E is a risk factor for the development of EMND, and the neurons supplying highly oxidative type I muscle fibers are primarily affected in EMND horses. Affected horses have low plasma concentrations of vitamin E. Horses with naturally occurring EMND require at least 18 months of a vitamin E deficiency before developing clinical signs, and an experimental model demonstrated that a 21-month interval of vitamin E deficiency was required before the development of disease. In addition, excessive dietary copper, a potential pro-oxidant, has been demonstrated to be a risk factor for EMND development.

In an experimental model of EMND, although all eight horses developed a vitamin E deficiency during the 30-month study, only four developed clinical signs of EMND. In naturally-occurring EMND, there appears to be an individual susceptibility to oxidative stress in at-risk horses, with clinical signs developing in only a subset of horses maintained in high-risk environments (i.e., no access to pasture and no vitamin E supplementation). It may be that the clinically unaffected horses are suffering from subclinical disease, as histologic lesions may be found in vitamin E deficient (but apparently unaffected) horses or it may be that specific genetic polymorphisms in genes involved in vitamin E metabolism determine individual susceptibilities to EMND under the same conditions of deficiency.

Horses without access to green forage are often supplemented with 1 IU/kg body weight/day of vitamin E (500 IU/500 kg horse) to prevent against EMND development. This dosage is similar to NRC requirements for horses without pasture access (600-800 IU/500 kg horse/day). For EMND-affected horses, supplementation with 5,000-7,000 IU alpha-tocopherol/day is recommended. This recommendation was made based on the synthetic formulation of alpha-tocopheryl acetate. With this treatment, approximately 40% of cases demonstrate clinical improvement within six weeks and many may appear normal within three months. It should be noted, though, that return to performance may result in deterioration. Approximately 40% of cases will stabilize but remain permanently disfigured, while 20% will have continual progression of clinical signs.

Vitamin E Deficient Myopathy

Some horses with clinical signs of EMND and a deficiency in vitamin E are not diagnosed antemortem with EMND because they lack evidence of neurogenic atrophy in the sacrocaudalis dorsalis (SC) muscle. While such horses may account for the previously reported 90% diagnostic sensitivity of muscle biopsy for EMND, a recent study suggests that many such undiagnosed cases may be the result of a specific muscle (myogenic) presentation of vitamin E deficiency.

A recent study identified eight adult horses with acute or chronic onset of muscle atrophy and weakness that did not have neurogenic atrophy in SC muscle. SC muscle contained characteristic abnormal moth-eaten staining pattern of mitochondria. This vitamin E deficient myopathy has likely been missed previously because formalin-fixed biopsy specimens are most often evaluated for a diagnosis of EMND and mitochondrial staining is not possible with this fixative. Unlike the paucity of motor neurons as seen with EMND, the observed generalized weakness in the horses with abnormal mitochondrial stains was suggested to be due to a reversible manifestation of skeletal muscle/mitochondrial oxidative stress associated with vitamin E deficiency.

All horses with abnormal mitochondrial staining and lacking evidence of neurogenic atrophy in skeletal muscle recovered completely after vitamin E therapy. Muscle alpha-tocopherol concentrations were low in all affected horses, but serum alpha-tocopherol was only low in six of eight horses. Vitamin E deficient myopathy may be an entity unto itself or a predecessor to development of EMND, but this distinction was not evaluated in the study because all horses successfully responded to vitamin E therapy, thereby precluding a postmortem examination.

Can you provide some background on supplementing horses with vitamin E?

Depending on the availability of grass and fresh hay, a horse may consume less than the daily-recommended amount of vitamin E, and therefore, alpha-tocopherol. In such cases, many alpha-tocopherol supplements are available for horses.

Synthetic. There are two types of synthetic vitamin E, all-rac-alpha-tocopherol and dl-alpha-tocopherol. All-rac-alpha-tocopherol acetate is accepted as the International Standard (1 mg = 1 international unit). Forms of synthetic vitamin E are available as powdered or pelleted supplements. The all-rac-alpha-tocopherol acetate form is highly dependent on adequate bile salts to generate hydrolysis and subsequent absorption of alpha-tocopherol into the plasma.

Natural. There are natural forms of RRR-alpha-tocopherol available for supplementation in horses. RRR-alpha-tocopherol is available as a micellized liquid form and as an esterified form (acetate). In order for alpha-tocopherol acetates to be utilized in the body, the ester has to be removed and the alpha-tocopherol made water-soluble by the action of bile salts (micellization).

The difference between natural and synthetic alpha-tocopherol in the horse was recently demonstrated, where CSF concentrations of alpha-tocopherol were significantly elevated above baseline values after supplementation with the natural alpha-tocopherol, but not following supplementation of synthetic at equivalent high dosages (10,000 IU/500kg horse/day). Increased CSF alpha-tocopherol concentrations have also been demonstrated with 5,000/ IU/500kg horse per day of natural alpha-tocopherol. In addition, although serum values of alpha-tocopherol increased significantly from baseline values with both the natural and synthetic form of vitamin E, serum alpha-tocopherol concentrations were significantly higher in the group supplemented with the natural compared to the synthetic. These values were approximately two times that of concentrations obtained using synthetic, which is in agreement with other studies showing that the activity of natural is between 1.36 and 2 IU/mg higher than synthetic. A more recent study showed that, with 5,000 IU/day/500 kg horse of powdered alpha-tocopheryl acetate formulations, it often takes 56 days or more to re-establish normal alpha tocopherol concentrations in the serum. Serum alpha-tocopherol drops rapidly when horses are switched from liquid alpha-tocopherol to powdered alpha-tocopheryl acetate at the same 5,000 IU/day/horse dose.

The argument for supplementing horses with the natural form of alpha-tocopherol arises from the knowledge that this form is the most biologically available, most readily absorbed, and has the most potent antioxidant activity.

Supplementation of Healthy Horses

In healthy young and middle-aged horses with adequate dietary vitamin E intake, research-based evidence for the need for or harmful effect of additional alpha-tocopherol supplementation (>500 IU/day) is scarce. Vitamin E, unlike other fat-soluble vitamins, does not accumulate in the body to a toxic level due to protective mechanisms. Excessive supplementation of alpha-tocopherol may increase plasma/serum and tissue concentrations to a certain level; however, there is strong evidence that tissues will become saturated and additional alpha-tocopherol will be metabolized and/or excreted.

In healthy exercising horses, high dosage of alpha-tocopherol supplementation (ten times the NRC requirement) was shown to be potentially detrimental to beta-carotene absorption, and this high dose was not recommended. Healthy horses should receive the NRC dietary recommendation of vitamin E. Additional supplementation above this requirement does not appear necessary in healthy horses.

Supplementation of Horses with Neurologic Disease

Many publications recommended high levels of vitamin E supplementation for horses with neurologic disease, ranging from 1,500 IU to 12,000 IU/500 kg horse/day. In horses with equine protozoal myeloencephalitis, supplementation with 5,000

IU/adult/day is recommended. In cases of cranial trauma, 20,000 IU/adult/day is recommended. In horses with cervical stenotic myelopathy, vitamin E is recommended at three times the NRC dose. Note that these studies do not necessarily stipulate the formulation of vitamin E being recommended.

The use of vitamin E for these conditions is empirical based on a belief that it may be neuroprotective in disorders not related to a deficiency of vitamin E. It is important to consider that many of these dosage recommendations exceed the NRC upper safety recommendation of 20 IU/kg (10,000 IU/500 kg horse). Furthermore, many of these studies were performed prior to the development of natural forms of vitamin E in horses, and these amounts may well be excessive if natural vitamin E is used therapeutically. At this time, there is no scientific evidence that supplementation with doses of alpha-tocopherol above the NRC-recommended dose will have a therapeutic effect in horses suffering from neurologic diseases other than those associated with a vitamin E deficiency.

The goal of alpha-tocopherol supplementation in horses predisposed to or affected by eNAD/EDM, EMND, and vitamin E deficient myopathy is to increase the concentration of alpha-tocopherol in the central nervous system or muscle tissue. In humans, alpha-tocopherol concentrations in CSF can be assessed by measures of serum levels, as they are highly correlated. Modest increases in the CSF alpha-tocopherol concentrations have been demonstrated in humans supplemented with alpha-tocopherol for an average of 332 days, and this modest increase was consistent with striatal brain alpha-tocopherol concentrations in supplemented mice.

In horses, only short-term studies of CSF have been performed. One study supplemented horses with RRR-alpha-tocopherol at 1,000 IU/500 kg horse/day (within NRC requirement) for 10 days and found significantly increased serum alpha-tocopherol concentrations compared to baseline values but did not find a significant increase in CSF alpha-tocopherol concentrations. Even with a tenfold higher dose for the same time period, there was not a significant increase in CSF alpha-tocopherol concentrations, although a 1.3- to 3.4-fold increase was noted in 9/10 horses. When 10,000 IU/500 kg horse/day was provided as RRR-alpha-tocopherol for an additional 14 days, a significant increase in CSF alpha-tocopherol was apparent. Supplementation with 5,000 IU/500 kg horse/day over two months also showed significant increases in CSF alpha-tocopherol.

Supplementing eNAD/EDM horses with 5,000 IU/450 kg horse/day provided clinical improvement in some cases; however, none of these reported cases returned to normal. More recent evidence has found no effect with vitamin E supplementation on neurologic improvement in affected horses. For EMND horses, it is recommended to supplement with 5,000–7,000 IU alpha-tocopherol/day/450 kg horse. With this treatment, approximately 40% of cases demonstrate clinical improvement within six weeks, and many may appear normal within three. With supplementation, concentrations of alpha-tocopherol in the CSF of eNAD/EDM cases are comparable to age-matched controls on similar supplementation protocols. Therefore, alpha-tocopherol is effectively transported into the central nervous system of eNAD/EDM cases. However, even with this increase in CSF alpha-tocopherol concentrations, clinical improvement is unlikely with eNAD/EDM.

Recommended Reading by the Authors

The information provided in this paper was extracted from the following article:

Finno, C.J., and S.J. Valberg. 2012. A comparative review of vitamin E and associated equine disorders. J. Vet. Intern. Med. 26:1251-1266. ✦

Advances in the Understanding of Gastric Ulcers in Horses

Al Merritt, D.V.M., Diplomate A.C.V.I.M.

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For background, why did you choose to study gastric ulcers? What attracted you to this specific disease?

During my time in the mid-1960s as an instructor at New Bolton Center, at the University of Pennsylvania School of Veterinary Medicine, I would spend one day every couple of weeks at the Penn State campus in Philadelphia. Because there were no residencies in those days, I shadowed a clinical specialty of interest within the medical school. I became fascinated with gastroenterology and that culminated in being awarded a fellowship to study for two years with Frank Brooks, M.D., who was the head of the gastrointestinal section at the Hospital of the University of Pennsylvania and also held a professorship in the Department of Physiology.

Brooks and his group were doing a lot of research on the stomach, using dogs and rats as the primary models. Since I was a vet with special interest in large animals, he suggested that I might like to look at gastric secretion in the conscious miniature pig, which was an animal model of growing interest at the time. The subsequent findings were the first to characterize basal- and histamine-stimulated gastric secretion in that species. At the end of that fellowship, I returned to the faculty at New Bolton Center as an internist with a specialty interest in gastroenterology, on a fifty-fifty clinic and research appointment. The real focus on horses started then. Thus, as someone specializing in all aspects of equine gastroenterology with some specific post-veterinary-degree training in gastric physiology, attraction to the problem of gastric ulcers was a no-brainer.

When did you first begin to study or research gastric ulcers? At that time, what were the primary research interests in the field? What was happening?

My initial attraction to equine gastric ulcers was in the early 1980s, when I was at the University of Florida, but it was gastroduodenal ulcer disease (GDUD) in foals, an entirely different problem from that seen in adults, which induced the interest. GDUD was much more commonly seen in those days than it is now; the gastric lesions were confined to the nonglandular (squamous) mucosa, and in more serious cases, especially when duodenal stricture was present, extended up into the esophagus. There were herd outbreaks as well as individual cases, and there were some indications that the problem might be associated with rotavirus infection, though that was never fully established. The current outlook is that the primary lesions are in the duodenum, with secondary lesions in the stomach and esophagus due to gastric acid reflux. A definitive etiology has yet to be identified.

I think that the seminal report that brought the attention of gastric ulcers in adults to the equine world was by Hammond and coworkers that appeared in the *Equine Veterinary Journal* (EVJ) in 1986 concerning necropsy findings of gastric lesions confined to the squamous mucosa, but with no accompanying duodenal lesions, in retired Thoroughbreds in Hong Kong (Hammond et al., 1986). From this point on the answers to the questions posed will be confined to equine gastric ulcer syndrome (EGUS) in adults.

In those early years, what was considered state-of-the-art technology when it came to diagnosing ulcers? Was diagnosis based solely on clinical signs? What were those signs?

Once we knew gastric lesions could occur in horses, the push was on to apply endoscopy to make a definitive diagnosis. At that time, state-of-the-art technology in human medicine was fiber-optic endoscopy but, except for some colonoscopes which were very expensive, none of the commercial units was long enough for use in the horse. Nonetheless, various manufacturers soon had either units designed for humans, or prototypes being designed for horses, that were at least two meters in length that started to become available, but they still were not inexpensive. The milestone paper concerning fiber-optic gastroduodeoscopy in the horse, written by Chris Brown and colleagues, appeared in the *Journal of the American Veterinary Medical Association* in 1986 (Brown et al., 1986). Their specially made instrument was 2.75 meters in length.

As technology moved from fiber optic to digital imaging, longer scopes became commercially available, though still expensive, and improved markedly the imaging that could be easily stored in an accompanying processor or a computer for later analysis. Still, the focus early on was on lesions within the squamous mucosa for which a two-meter-long unit sufficed. This is the classic "gastric ulcer disease" described in the horse at that time, seen with high prevalence in animals in training and best characterized early on by Jack Snyder and Nick Vatistas in California and Mike Murray in the East. As more and more animals were scoped, however, it became clear that lesions could also occur within the glandular mucosa and that required the need of longer scopes for detailed observation. Most videoendoscopes now used in horses are three meters long, which allows for close inspection of the upper duodenum as well as the full stomach.

With respect to clinical signs, recognition of those that could be indicative of gastric ulcer disease resulted more from scoping animals that just "weren't right" than the other way around. That is, complaints of horses showing uncharacteristic irritability, failure to finish a meal, signs of mild abdominal discomfort, and failure to perform up to expectation were grounds for gastric endoscopy, and many of those animals had significant ulceration, most commonly of the squamous region. But as more and more endoscopies have been done over time for one reason or another, we now know that quite severe squamous lesions can also be present in animals that appear clinically normal.

It's important at this point to talk about terminology. As indicated above, of major clinical interest in gastric ulcers in adults during the late 1980s and most of the 1990s was those lesions occurring in the squamous mucosa and their effect on performance, especially since they seemed to have greatest prevalence in animals in training. Lesions of the glandular mucosa that were also seen from time to time were attributed little clinical significance except for those few that were associated with intensive NSAID therapy. Then, in 1999, a group of academic and private clinicians, of which I was one, who had been assembling annually for a number of years to share ideas and current research results concerning gastric ulcers under the auspices of Merial, published a consensus report regarding the current knowledge of the problem in *Equine Veterinary Education* (Andrews et al., 1999).

That report suggested classifying all of the various conditions that manifest as ulcerative lesions within the esophagus, stomach, or upper duodenum—sites with potential exposure to gastric acid—as "equine gastric ulcer syndrome" (EGUS). Further explanation of the term stated, "While the name does not adequately describe all manifestations of the syndrome, adaptation into conventional vocabulary suggests that the reference be maintained." From this, the EGUS term took off. However, it was often used with no qualification of whether it was referring to squamous or glandular lesions, or both, which implied that EGUS is one disease rather than it being a term that refers to a number of unique problems that manifest upper gastrointestinal ulceration.

Thus, in 2009, I wrote an editorial in the *Equine Veterinary Journal* titled "Appeal for the Proper Use of the Term "EGUS": Equine Gastric Ulcer Syndrome," in which I stated that it is important to include a qualifier concerning which particular form of problem falling within the EGUS domain is under consideration in any written or oral communication. "Only this will reinforce the very important concept that EGUS is not one disease, which is critical to promoting further accurate knowledge about these problems in the upper GI tract of the horse." That appeal was gratefully taken to heart by many of the current workers in the field, most notably Ben Sykes who was instrumental, along with some European colleagues, in publishing in 2015 in *Journal of Veterinary Internal Medicine* a consensus statement from the European College of Equine Internal Medicine promoting two subclassifications of the EGUS term: equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD).

In those days, what were the standard treatments for gastric ulcers?

Once EGUS was recognized, it was only natural to turn to therapeutic protocols similar to those being used in human medicine, most notably agents that either buffered or blocked secretion of gastric acid. It was pretty clear early on that effective antacid therapy promoted healing of ESGD and this was somewhat good fortune given what we now know about the major role of gastric acid in the pathogenesis of squamous ulceration, and the similarities between ESGD and human gastroesophageal reflux disease (GERD), for which the prime treatment is an antacid. Histamine-2 receptor antagonists were the antisecretory drug class of choice in human medicine in the 1980s and those that were available were, of course, formulated for human use and dosage. Were they effective in the horse, and at what dose?

In 1984, Martha Campbell-Thompson, a brilliant, resourceful vet who had finished a surgery residency and was then a graduate student of mine, developed a technique for chronic gastric Silastic cannulation of the horse that allowed collection of gastric contents from conscious animals over various time periods. With that model, we reported that intravenous ranitidine effectively blocked acid secretion for a number of hours. But the intravenous route was certainly not practical for routine use in the equine world, so homemade preparations of ground-up pills or powder in some vehicle like commercial syrup were devised, and through various studies and evidence-based experience, a dose of 6.6 mg/kg of ranitidine three times a day was settled upon, considerably higher than that recommended for humans. An excellent paper by Mike Murray and Gerald Schusser in the *Equine Veterinary Journal* expressed the culmination of the oral ranitidine experience where they monitored intragastric pH via an indwelling catheter in conscious horses before and during treatment. It showed a reasonable effectiveness but, again, required administration three times a day (Murray and Schusser, 1993).

Other agents tried early on included the PGE2-inducing compound sucralfate and other substances that presumably provided a protective coating of the squamous mucosa against acid damage, but these never took hold in a big way. The same for orally-dosed buffering agents, which we later showed, in cannulated animals, had only a very short-term effect (Clark et al., 1996).

Then came omeprazole, with the first studies of acid inhibitory effect in cannulated horses reported in the *Equine Veterinary Journal* by Andrews and coworkers (1992) and Christine Jenkins and coworkers (1992). Jenkins learned the cannulation technique from Martha Campbell-Thompson. We subsequently worked with Merial, using our herd of cannulated horses at the Island Whirl Equine Colic Research Laboratory at the University of Florida, in the development of an oral paste formulation of omeprazole that was finally marketed as GastroGard®. Then, Andrews led the critical trial of GastroGard® that verified its anti-ESGD effects in horses in training, which was reported in the *Equine Veterinary Journal* in 1999 (Andrews et al.). While this product was considerably more expensive than ranitidine, who could argue against a paste formulation that only needed to be given once a day? The rest is history.

In looking specifically at the work you've done, what has changed in the way you study the disease? Specifically, how has technology paved the way to more accurate diagnosis?

Since I retired in 2003, I have not been personally involved in studying the disease for some time now. But technologically speaking, I think it is safe to say that endoscopy is still the gold standard of accurate diagnosis and determination of disease severity and extent. That said, Hewetson and coworkers in Noah Cohen's lab at Texas A&M University have verified a sucrose permeability technique, which involves measuring sucrose in serum collected sequentially after intragastric sucrose installation, as a less invasive method for screening a group of animals than endoscopy, though it still requires nasogastric intubation to instill the sucrose (2006). There is also a kit on the market for semi-quantitative measurement of hemoglobin in the feces, but its accuracy in diagnosing gastric ulcer disease has not been verified to my knowledge.

The big technical leaps have involved the endoscopes themselves. There are now reasonably priced three-meter videoendoscopes on the market that can be plugged into a laptop computer via a USB connector, thus miniaturizing all that previously ponderous imaging and storage gear. The associated software allows for all sorts of image projection, enhancement, and manipulation that was not previously possible.

Your accomplishments in understanding, researching, and reporting on gastric ulcers cannot be undervalued. What do you feel are your greatest achievements in this area?

I cannot answer this question without first acknowledging the collaboration of some wonderful colleagues, graduate and veterinary students and technicians who really were the ones who made it all work. With respect to achievements concerning the understanding of ulcer disease in adults, here are the reports that I think are most notable:

Campbell-Thompson, M.L., and A.M. Merritt. 1987. Effect of ranitidine on gastric secretion in young male horses. Am. J. Vet. Res. 48:1511-1515. (As mentioned above, this was the first study of the effect of a drug on gastric acid secretion in the conscious gastrically cannulated horse.)

Campbell-Thompson, M.L., and A.M. Merritt. 1990. Basal- and pentagastrin-stimulated gastric secretion in young horses. Am. J. Physiol. 259:R1259-1266.

(This was groundwork characterization of gastric acid secretory physiology in the horse.)

Lorenzo-Figueras, M., and A.M. Merritt. 2002. Effect of exercise on equine proximal intragastric pressure and pH. Am. J. Vet. Res. 63:1481-1487. (This study, involving intragastric pH and barostat probes, provided one explanation of why horses in training have such a high incidence of ESGD. Basically, tensing of the abdominal muscles, which occurs when horses execute a gait faster than a walk, causes increased intra-abdominal pressure. This, in turn, causes increased intragastric pressure that pushes acidic gastric content up into the squamous region which, when the horse is at rest, normally has a rather high pH.)

Merritt, A.M., L.C. Sanchez, J.A. Burrow, M. Church, and S. Ludzia. 2003. Effect of GastroGard® and three compounded oral omeprazole preparations on 24 hour intragastric pH in gastrically cannulated horses. Equine Vet. J. 35:691-695. (This study showed that how omeprazole is compounded has major effects on how efficacious it is. It also showed the relatively superior efficacy of GastroGard®, which worked more quickly but not quite as long as previously assumed.)

Cargile, J.L., J.A. Burrow, I. Kim, N.D. Cohen, and A.M. Merritt. 2004. Effect of dietary corn oil supplementation on equine gastric acid, sodium and PGE2 content before and during pentagastrin infusion. J. Vet. Internal Med. 18:545-549. (This study confirmed the prostaglandin-E2 stimulating properties of corn oil. PGE2 is important in maintaining the acid barrier of the gastric glandular mucosa.)

Husted, L., L.C. Sanchez, S.N. Olsen, K.E. Baptiste, and A.M. Merritt. 2008. Effect of paddock versus stall housing on 24-hour gastric pH within the proximal and ventral equine stomach. Equine Vet J. 40:337-341. (This work was actually under the direction of Chris Sanchez, who had been one of my graduate students. It provided important baseline data concerning the effect of feeding and housing on the pH of contents in the top and bottom of the stomach. Among other things, the results suggested that, with respect to ESGD pathogenesis, meal feeding may not be the best management plan.)

Merritt, A.M. 2009. Appeal for proper usage of the term "EGUS": Equine Gastric Ulcer Syndrome. Equine Vet. J. 41:616. (This is the editorial I mentioned above.)

7. What work have other researchers done in this area that you feel will have a lasting impact?

Jack Snyder and Nick Vatistas: Early leaders in characterizing ESGD and its prevalence by endoscopic studies at Thoroughbred tracks in California.

Mike Murray: The ultimate endoscopist who led the way in characterizing both ESGD and EGGD in adults and foals. His findings are classic and, for the most part, enduring. He also made important contributions to our knowledge of the pathophysiology and treatment of various manifestations of EGUS. I cannot say enough about Mike's contributions.

Frank Andrews: Along with Jennifer Nadeau and others in his group, he drew our attention to the corrosive properties of some volatile fatty acids, which are by-products of intragastric fermentation of soluble carbohydrate feedstuffs, on the squamous mucosa when there is gastric acid present. This has important implications for prophylactic feeding strategy. As mentioned above, he led the field trial that confirmed the therapeutic effectiveness of GastroGard® on ESGD and has continued to explore alternative ways of treatment and prophylaxis, including various nutraceuticals.

R.J. Bell: His endoscopic studies in New Zealand were the first to show that there can be a notable prevalence of ESGD in sedentary animals, thus questioning the prevailing outlook that it was primarily a problem in athletes.

Nana Luthersson: She has done some classic epidemiological studies of ESGD and EGGD in both athletic and sedentary horses in Europe. Among other things, her findings indicate a strong relationship between high grain feeding and the prevalence of both ESGD and EGGD.

Ben Sykes: As indicated above, he was instrumental in promoting the subclassification of EGUS into ESGD and EGGD, which is critical to how these problems will be reported and studied in the future. He has also done very important studies of the efficacy of various forms and formulations of omeprazole.

How do you think researchers will approach gastric ulcers in the future?

With respect to ESGD, the basic pathophysiology is pretty well understood—excessive acid exposure. One question is how much diet, especially concentrates, contributes to this, as indicated in the reference to the work of Andrews, Nadeau and coworkers, and Luthersson, mentioned above. Dietary manipulation combined with the application of various problem-oriented nutraceuticals and other management factors designed to reduce the incidence in lieu of pharmaceuticals is where the challenges lie. Scientific documentation is essential.

With respect to EGGD, except for the occasional association with excessive NSAID therapy, the cause(s) is unknown. Based on other species, a Helicobacter infection seems like an obvious culprit, but neither this organism nor any other microbe has yet been documented as an etiological agent. Until the etiology is identified, no specific therapy or management can be applied. It is known that omeprazole therapy is not as efficacious for treating EGGD as it is for ESGD, indicating that the basic cause is not simply too much exposure to gastric acid.

During your career, what other areas of study were you interested in?

Other areas of study in equine gastroenterology have been:

- Causes, consequences, and clinical management of chronic diarrhea
- Xylose absorption in normal adults and foals and those with diarrhea and weight loss
- Characterization of gastrointestinal motility and effects of various drugs on motility and transit
- Effect of diet on gastric motility and emptying
- Effect of acupuncture in ameliorating discomfort in a mild, controllable colic model

Can you suggest two or three review papers or book chapters that would provide an overview of the subject?

Merritt, A.M. 2003. The equine stomach: A personal perspective (1963-2003). Milne Lecture. In Proc. American Assoc. Equine Prac. 49:75-102.

Sykes, B.W., M. Hewetson, R.J. Hepburn, N. Luthersson, and Y. Tamzali. 2015. European College of Equine Internal Medicine Consensus Statement—Equine Gastric Ulcer Syndrome in Adult Horses. J. Vet. Intern. Med. 29:1288-1299.

Camacho-Luna, P., B. Buchanan, and F.M. Andrews. 2017. Advances in diagnostics and treatments in horses and foals with gastric and duodenal ulcers. Vet. Clin. Equine 34:97-111.

Do you have any other remarks about gastric ulcer disease in horses?

I think it's safe to say that mild ESGD, small areas of superficial erosions near the margo plicatus, probably falls within the realm of normality for all equids, because that squamous region lies closest to gastric contents that are usually $<$pH 4.0, which is generally considered the corrosive cutoff. The more severe ESGD lesions, those that extend well up into the squamous region and may bleed, and most likely all EGGD lesions, are more likely due to human-determined conditions, such as forced exercise, feeding strategies, housing conditions, increased exposure to infectious agents (for some cases of EGGD?), and undoubtedly other factors we have not yet thought of.

Man and horse have a long history of close interaction which, save for some future unimaginable situation, ain't gonna change. But hopefully future management modifications away from dependence on pharmaceuticals that do not impact on the quality and expectations of that interaction can be devised that result in a significant reduction in the incidence of all forms of EGUS, for the sake of both man and beast.

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Hammond C.J., D.K. Mason, and K.L. Watkins. 1986. Gastric ulceration in mature Thoroughbred horses. Equine Vet. J. 18:284-287.

Hewetson, M., N.D. Cohen, S. Love, R.K. Buddington, W. Holmes, G.T. Innocent, and A.J. Roussel. 2006. Sucrose concentration in blood: A new method for assessment of gastric permeability in horses with gastric ulceration. J. Vet. Intern. Med. 20:388–394.

Murray, M.J, and G.F. Schusser. 1993. Measurement of 24-h gastric pH using an indwelling pH electrode in horses unfed, fed and treated with ranitidine. Equine Vet. J. 25:417-421. ✦

Advances in Vitamins and Minerals

Laurie Lawrence, Ph.D.

Why did you choose to study equine nutrition? What attracted you to this field of study?

I grew up on a small horse farm where regular chores included feeding various classes of horses (lay-ups, broodmares, foals, and yearlings). I was exposed to different types of hay and grains, and I always wondered why we would make changes to the diets of different horses. When I went to Cornell, I was assigned to Dr. Harold (Skip) Hintz for advising. His research was really interesting to me, and I had the opportunity to work with one of his graduate students.

As background, can you tell me a little about the time when researchers began looking at vitamin and mineral requirements in horse diets? Who were the pioneers in this field?

From old textbooks you can see that animal scientists and veterinarians recognized that some clinical abnormalities were associated with certain feeding practices. Because feed analysis was not easy then, the actual nutrients involved in the abnormalities were not always known. Once assays became more available, research started to increase. Of course, horse research lagged far behind swine and poultry in the mid-1900s because horses were no longer important for farming.

In the late 1960s, researchers in the U.S., like Dr. Skip Hintz, Dr. Herbert Schryver and Dr. Howard Stowe, began to look at minerals and vitamins. I never met Dr. P.A. Linerode, but he was one of the few researchers to look at B-vitamins. There were also a number of scientists outside of the U.S. that published important data about vitamins and minerals.

When did you first begin to study or research vitamins and minerals? At that time, what were the primary research interests in the field? What was happening?

In college, I wrote a report about supplements, which were starting to gain popularity then. We take commercially manufactured fortified feeds as a given in horse feeding today but, in the 1960s and 1970s, they were not all that common.

In the same vein, what was considered state-of-the-art technology when it came to determining digestibility of nutrients? What equipment, for example?

A few studies used collection harnesses but straight stalls that allowed collection of urine and feces from geldings were pretty common. If you look at some of the older papers, collection periods of seven days or longer were not uncommon, compared to what you see today where some published studies have used very short collection periods.

In looking specifically at the work you've done, what has changed in the way you study vitamins and minerals? How has technology changed the way to measure digestibility?

I think we understand more about how nutrients, especially minerals, are metabolized post-absorption, particularly how minerals that are absorbed in excess of need are excreted from the body.

How has the work that you and your peers accomplished in this area furthered the understanding vitamin and mineral nutrition?

At the conference, I will talk about some of the research studies that Dr. Ashley Fowler and Dr. Mieke Brummer-Holder have conducted at the University of Kentucky on phosphorus and selenium, respectively.

What does the future hold for vitamin and mineral exploration in equine nutrition?

A lot of research is limited by cost. Currently, it is expensive and complicated to analyze most vitamins, so our understanding of vitamin nutrition will probably improve slowly. On the other hand, I expect that concerns about environmental impacts of mineral excretion will drive some studies to more accurately identify requirements to prevent overfeeding.

During your career, what other areas of study have you become interested in? Have you been able to pursue those?

Initially, a lot of my research pertained to feeding the performance horse. Because of some infrastructure changes in our research facilities, I moved away from that to pursue nutrition related to mares and foals. Recently we have conducted a number of studies aimed at understanding the development of digestive processes in the growing horse. In general, we assume that they digest feedstuffs just like mature horses and that may or may not be true. I have also had the opportunity to expand my knowledge of forages and forage utilization. The University of Kentucky has a great group of forage scientists in the Plant and Soil Science Department and it has been fun to learn from them.

In lieu of a reference list, can you suggest two or three review papers or book chapters that would provide an overview of the subject?

This one comes to mind:

Lawrence, L. 2011. Nutrition for the broodmare. In: A.O. McKinnon, E.L. Squires, W.E. Vaala, and D.D. Varner, editors, Equine reproduction. Wiley-Blackwell, Ames, IA. p. 2760-2768.

Do you have any other closing remarks?

We have an embarrassment of riches right now in the range of commercial feeds available for horses. We take for granted that we can walk into a feed dealer and purchase a fully fortified feed formulated for almost any type of horse, and possibly formulated for a horse when it is fed a specific forage, or no forage at all. These are all innovations that have occurred since the 1960s. It is interesting to me that, despite the levels of fortification in commercially manufactured feeds, the use of additional supplements containing vitamins and minerals is still a common practice. ✦

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On the following pages, you will find summaries of the work of the work carried out by Kentucky Equine Research's technical staff over the years, grouped by decade.

Maternal-Offspring Bond of Ponies Fed Different Amounts of Energy

K. Houpt, H.F. Hintz, and J.D. Pagan

This study was designed to find the effect of varying the caloric level of the maternal diet during lactation on the response of 23 mares and their suckling foals to separation.

Pony mares were fed 9.33, 7.48, or 5.75 Mcal digestible energy/100 kg/day, which corresponded to 128, 103, or 79% of the National Research Council (1978) requirement, respectively. Foals were not allowed access to solid food and obtained all their nutrients from the dam's milk. The behavior of the mare in her home stall with the foal was compared with her behavior when the foal was removed for a 5-minute period.

Diet had no effect on the maternal response, even though the mares fed the low-energy diet lost weight and the other groups gained weight. Results showed that due to separation the mare became less attached to the foal as it matured. Undernutrition had little effect on the mares' response to separation. Defecation, pawing, and snorting by the mare and foal increased during periods of separation. An unexpected finding was that a foal was not active in the absence of its mother during the first week of life.

This research was published in *Nutrition and Behavior*, 1983. ✦

Digestible Energy Requirements of Lactating Pony Mares

J.D. Pagan and H.F. Hintz

Lactating pony mares were fed one of three rations for 12 weeks. The rations provided 80, 100, or 125% of digestible energy requirements, based on NRC (1978) recommendations. All diets provided 100% of protein requirements and adequate calcium and phosphorus. Mares and foals were weighted weekly, and foal heart-girth and wither height were also measured weekly.

Mares receiving the low-energy diet lost weight and their foals gained weight more slowly and had smaller heart girths, though they were as tall as the other foals. Mares on the medium and high energy diets gained weight at similar rates. Their foals also gained weight and grew at similar rates.

This was published in *Journal of Animal Science*, 1984. ✦

Equine Energetics I: Relationship Between Body Weight and Energy Requirements in Horses

J.D. Pagan and H.F. Hintz

Short title: Do larger horses need a lot more feed than smaller horses?

In this study, researchers conducted a series of experiments in which the resting maintenance energy requirements of equids varying greatly in body size were measured. Four mature animals of 125 kg, 206 kg, 500 kg, and 856 kg were fed three different levels of intake and the net amount of energy retained at each level was determined. The individual's resting maintenance requirement was then determined by regression analysis of energy intake against energy balance. The level of energy intake that would result in zero energy balance was considered to be the animal's maintenance energy requirement. The results of this study suggest that maintenance energy requirements of equids vary linearly with body weight.

This research was published in *Journal of Animal Science*, 1986.✦

Equine Energetics II: Energy Expenditure in Horses During Submaximal Exercise

J.D. Pagan and H.F. Hintz

This experiment was conducted to measure the amount of energy expended by horses traveling at speeds up to 400 m/minute on a racetrack both with and without a rider, and to attempt to use these data to formulate feeding standards for horses performing submaximal exercise.

Four geldings were used in the study. The horses exercised on a track, both with and without a rider, and expired air was collected using a face-mask that allowed energy expenditure to be measured with a mobile modified open circuit indirect respiration calorimeter.

For all horses, more energy was expended when horses carried a rider. The amount of energy was related to speed and was also proportional to the body weight of the animal or the combined weight of the horse and rider. Fat is known to be the primary substrate for energy generation in horses during low intensity work. Using a value of 55 to 60% for the efficiency of utilization of digestible energy for submaximal work, these values can be used with a supplied equation to calculate the additional amount of digestible energy needed by horses working at this level.

This research was published in *Journal of Animal Science*, 1986. ✦

The Effect of Dietary Energy Source on Exercise Performance in Standardbred Horses

J.D. Pagan, B. Essen-Gustavsson, A. Lindholm, and J. Thornton

Three Standardbred horses were used in a 3 x 3 Latin square design trial to determine the effect of feeding diets containing different levels of carbohydrate, fat, and protein to exercised horses. During each one-month period the horses were fed either a 12% crude protein (CP) (as-fed basis) commercial horse feed (control), a 20% CP feed (high-protein diet), or an 11% CP feed containing 15% added soybean oil (high-fat diet). During week 3 of each period, the horses performed a stepwise exercise test and a high-speed exercise test on a flat motor-driven treadmill.

During week 4 of each period, the horses performed a long, slow exercise test (105 min at 5 m/sec). Muscle and liver glycogen concentrations were significantly lower after the long, slow exercise test when the horses were fed the high-protein and high-fat diets than when fed the control diet. Respiratory quotients were also significantly lower than control values during submaximal exercise when the horses were fed the high-protein and high-fat diets. Type of diet affected the glycogen storage and metabolic response to exercise. Horses fed the high-protein and high-fat diets appeared to respond similarly.

This research was published in *Proceedings of the 2nd International Conference on Equine Exercise Physiology*, 1987. ✦

The Oxygen Cost of Weight Loading and Inclined Treadmill Exercise in the Horse

J. Thornton, J.D. Pagan, and S. Persson

This study was carried out to determine the metabolic response to load carrying and inclined trotting during treadmill exercise in the horse.

Five Standardbred trotters trained for treadmill exercise were initially subjected to an incremental exercise test, without and then with a load of approximately 10% body weight in the position of a riding saddle. Each horse was then run on the flat and subsequently at a 6.25% slope at speeds known to be of an aerobic intensity. Oxygen consumption (VO_2), stride frequency, heart rate, and respiratory rate were recorded while each horse ran for 5 minutes without the load and then 5 minutes with the load.

The additional load did not increase the oxygen cost per kgm of the exercise and the ratio of increase in VO_2 to increase in total mass was 1.04 ± 0.07 and 1.08 ± 0.11, respectively for horizontal and inclined exercise. Inclined trotting without the load was metabolically more expensive than load carrying horizontally or uphill due to an oxygen cost of ascent of 1.379 ml O_2/kgm. The increased oxygen requirements were met largely by an increase in ventilation until a maximal tidal volume was approached. It was concluded that oxygen uptake during load carrying increases proportionally to the load during horizontal and inclined exercise, and that inclined exercise requires a greater increase in VO_2 than does the addition of a load during either horizontal or inclined exercise.

This research was published in *Proceedings of the 2nd International Conference on Equine Exercise Physiology*, 1987. ✦

Composition of Milk from Pony Mares Fed Various Levels of Digestible Energy

J.D. Pagan and H.F. Hintz

Twenty-two pony mares were fed one of three diets that provided 93.0, 74.8 or 57.2 kcal of digestible energy (DE) per kg body weight per day. Milk samples were taken at 14-day intervals. A total of five samples were taken from each mare. The samples were analyzed for total solids, crude protein, lactose, total lipids, ash, calcium and phosphorus. Gross energy was calculated from composition data.

Increases in energy intake decreased the concentration of total solids, protein, fat, and gross energy of mare's milk. Energy intake had a greater influence on the mare's body condition than on milk energy production. It was concluded that the objective of a feeding program for a lactating mare should be to keep the mare in a desirable body condition rather than to influence milk composition or production.

This research was published in *Cornell Veterinarian,* 1986. ✦

The Effect of Dietary Energy Source on Blood Metabolites in Standardbred Horses During Exercise

J.D. Pagan, B. Essen-Gustavsson, A. Lindholm, and J. Thornton

This study was conducted to determine whether changes in dietary energy source (carbohydrate, protein, and fat) influence substrate selection by equine muscle during different exercise intensities. The study concentrated specifically on the effect of dietary energy source on blood metabolites in horses during exercise.

Three Standardbred horses were fed diets high in either carbohydrate, protein, or fat along with a 50% dry matter rye grass haylage. The horses were lightly trained on a treadmill for two weeks. In the third week the horses performed a high-speed exercise test on a treadmill and muscle biopsies and blood samples were taken. During the fourth week they performed a long slow exercise test on a treadmill and muscle, liver, and blood samples were taken.

Diet affected the substrates selected by the horses during both the fast and slow exercise tests. In all treatment groups, there was a sharp decline in blood glucose after the onset of exercise. It appeared that either high-fat or high-protein/low-carbohydrate diets resulted in a sparing of muscle glycogen utilization during exercise.

This research was published in *Proceedings of the 10th Equine Nutrition and Physiology Society Symposium*, 1987. ✦

The Effect of Exercise and Diet on Muscle and Liver Glycogen Repletion in Standardbred Horses

J.D. Pagan, B. Essen-Gustavsson, A. Lindholm, and J. Thornton

Two experiments were conducted to determine the effect of exercise and diet on muscle and liver glycogen repletion in horses.

In the first experiment, four Standardbred horses were fed either a high-carbohydrate or high-fat diet in a two-period switchback pattern. During the first 12 days of each period, the horses were fed their diets along with timothy hay and were allowed to exercise in small paddocks. The next phase was six days of exercise on a treadmill for increasing periods of time, with feed amounts rising as exercise increased. Muscle and liver biopsies were taken before and after the exercise phase. In the second experiment, three Standardbred horses were fed separate diets high in either carbohydrate, fat, or protein. Muscle biopsies were taken before and immediately after a strenuous bout of exercise. The horses were then allowed to rest for 7 to 10 days in stalls with some free exercise in small paddocks, after which muscle biopsies were again taken.

In the first experiment there was a 20% decrease in glycogen stores, and muscle glycogen concentration tended to be slightly lower than pre-resting values even after three days of rest with both diets. Liver glycogen was fully restored in both treatments. In the second experiment, horses on the high carbohydrate diet had muscle glycogen depleted by 29% by a strenuous exercise bout and then increased to 122% of the pre-exercised values.

This research was published in *Proceedings of the 10th Equine Nutrition and Physiology Society Symposium*, 1987. ✦

Influence of Copper Supplementation and Pelleted vs. Extruded Concentrate on Growth and Development of Weanling Horses

M.L. Thomas, E.A. Ott, J.D. Pagan, P.W. Poulos, and C.B. Ammerman

In this study, 22 Thoroughbred and Quarter Horse weanlings were fed three levels of copper supplementation and two forms of concentrate, extruded and pelleted, in a 112-day feeding trial. Bermudagrass hay was also fed. The weanlings were fed to appetite during two feeding periods each day. Copper supplements in pelleted form were fed prior to the regular portion of concentrate. Gains in height, weight, girth, and length were recorded, as were feed and hay intakes and feed efficiency.

Copper supplementation level did not influence weight, height, girth, or length gains. There was no influence of copper supplementation level on joint condition. There was no difference in bone density between groups. There were some early differences in weight gain by concentrate form, but these decreased with time. Serum analysis was not indicative of level of bone mineralization. Bone density analysis did not reveal differences between levels of supplemental copper.

This research was published in *Proceedings of the 10th Equine Nutrition and Physiology Society Symposium*, 1987. ✦

Influence of Isocaloric High Energy Carbohydrate and Fat Diets on Growth Related Hormone Profiles in the Yearling Horse

L. Lawrence, J.D. Pagan, M. Publos, J. Reeves, K. White, R. Douglas, and C. Gaskin

Hormones such as insulin, thyroxin, triiodothyronine, and cortisol control growth in young horses. Nutrition influences metabolic pathways and may affect hormone profiles. The objective of this study was to determine whether high-carbohydrate or high-fat diets affect certain hormone profiled associated with growth in horses.

In the study, 11 Thoroughbred, Quarter Horse, and Arabian horses aged 9 to 10 months old were assigned to one of two groups and were fed either high-energy (high carbohydrate) or high-fat diets containing the same caloric levels. Soybean oil was added to a basal diet for the high-fat group. Alfalfa hay was also fed. The horses were evaluated for obvious growth abnormalities at the beginning, midpoint, and end of the trial. They were weighed and measured weekly. Venous blood samples were taken before feeding and at 30 and 90 minutes after eating.

There was no difference in hip height, wither height, or heart girth related to diet. There was no visual evidence of bone development problems. In phase 2, glucose concentrations were higher for the high-carbohydrate diet than for the high-fat diet. Average insulin concentrations were also higher for the high-carbohydrate than for the high-fat diet in the second phase. Cortisol concentrations were lower in the high-fat groups before feeding. Horses moving from the high-fat diet to the high-carbohydrate diet showed higher intakes and higher weight gains.

It is known that elevated glucose and insulin levels in growing horses have the potential of dramatically affecting growth. More study is required before dietary manipulation of growth in horses can be achieved.

This research was published in *Proceedings of the 11th Equine Nutrition and Physiology Society Symposium*, 1989. ✦

Effect of Yeast Culture Supplementation on Nutrient Digestibility in Mature Horses

J.D. Pagan

Two experiments were carried out to evaluate the effect of live yeast culture supplementation on nutrient digestibility in horses.

Four mature Thoroughbred and Quarter Horse geldings were used in the studies. In the first experiment, the horses were fed fescue hay and a commercial sweet feed in two equal feeding per day with or without the inclusion of 10 g/day of yeast culture. Hay, feed, and feces were analyzed for dry matter, fiber, minerals, and other content.

Yeast culture supplementation increased digestion of dry matter, magnesium, potassium, phosphorus, and ash. The second experiment was similar except that dicalcium phosphate or wheat middlings were added as sources of phosphorus. Estimated true phosphorus digestibility was increased from 19.6% in the control diets to 24.1% in the yeast culture supplemented diets.

This research was published in *Journal of Animal Science*, 1990. ✦

Digestibility of Long-Stem Alfalfa, Pelleted Alfalfa or an Alfalfa/ Bermuda Straw Blend Pellet in Horses

J.D. Pagan and S.G. Jackson

This study was designed to find the effect of pelleting on the nutritional value of alfalfa hay and also an alfalfa/Bermuda blend.

Four mature horses were used in a series of digestion trials. In the first trial, the horses were fed pelleted or long-stem alfalfa from the same cutting. In the second trial, all horses were fed a pelleted blend of alfalfa and Bermuda straw. Analyses were made of the hay or pellets and the manure that was collected. Apparent digestibilities were determined for each nutrient by subtracting the total amount of nutrient excreted in the feces from the total intake of that nutrient during the collection period, assuming a constant amount of endogenous loss from each horse daily.

There was no significant difference between the digestibilities of any of the nutrients in the long-stem or pelleted alfalfa with the exception of fat, which was more digestible in the long-stem hay. Total digestible nutrients, dry matter, crude protein, and acid detergent fiber digestibility were significantly higher in the alfalfa than in the alfalfa/Bermuda blend. There was no significant difference between the digestibilities of any of the minerals in the long-stem versus pelleted alfalfa. Digestibilities of calcium and copper were higher in the alfalfa/Bermuda blend than in either the long-stem or alfalfa hay.

Pellets were completely consumed, while the long-stem hay tended to shatter and produce large quantities of dust and fines, which could lead to wastage under normal feeding conditions. Horses eating long-stem hay also produced wetter manure. This water loss could be a factor for horses living in hot climates.

This research was published in *Proceedings of the 12th Equine Nutrition and Physiology Society Symposium*, 1991. ✦

Distillers Dried Grains as a Feed Ingredient for Horse Rations: A Palatability and Digestibility Study

J.D. Pagan and S.G. Jackson

A series of preference tests and digestion trials were conducted to evaluate the suitability of distillers dried grains with solubles (DDG/S) as a feed ingredient for horses.

First, six horses were used in a 6 x 6 Latin square design two-choice preference trial to determine the effect that DDG/S have on the palatability of horse feed. Four pelleted diets were formulated to contain similar nutrient concentrations and either 0, 5, 10, or 20% DDG/S. During each period, two-choice preference tests were conducted for six consecutive days. The horses showed no preference between the pellets containing 0, 5, or 10% DDG/S. The 20% DDG/S pellet was preferred over each of the other pellets.

Following the preference trial, four horses were used in a 4 x 4 Latin square design digestion trial to evaluate the digestibility of the four diets. There was a trend toward decreased protein digestibility as the level of DDG/S was increased in the diet, but the difference was only statistically significant between the control and 20% DDG/S diet. Dry matter digestibility was slightly depressed in the 5% and 10% DDG/S diets, but fat digestibility and total digestible nutrient percent were not different between treatments. DDG/S appears to be a suitable ingredient for horse feeds at a level of inclusion of 20%.

This research was published in *Proceedings of the 12th Equine Nutrition and Physiology Society Symposium,* 1991. ✦

The Long Term Effects of Feeding Fat to 2-Year-Old Thoroughbreds in Training

J.D. Pagan, I. Burger, and S.G. Jackson

A study to evaluate the long-term effects of feeding a fat supplemented diet during medium intensity aerobic training was conducted over 7 months using 12 two-year-old Thoroughbreds.

Six horses in the control group were fed grass hay and a fortified sweet feed (CON), and six horses in the other group (FAT) received hay, sweet feed, a supplement pellet, and 400 ml of soybean oil that supplied about 12% of the dietary energy intake of the FAT group. After 2, 4, and 7 months, the horses performed two standardized exercise tests (SET) on a high-speed treadmill. The first test (STEP) consisted of sequential steps of 800 m at speeds of about 4, 8, 9, 10, and 11 m/s.

Oxygen consumption and lactate level increased with training, but were not different between treatment groups. During the last STEP, insulin was higher post feeding in the CON horses. Blood glucose was lower at the end of the STEP in CON horses. The second test (SET30) consisted of 30 min of trotting at about 4 m/s. Cortisol was elevated during exercise in the CON horses during the last SET30. Thyroxine, a thyroid hormone, was unaffected by diet or exercise. Consuming a fat supplemented diet altered the insulin response after feeding and this may have prevented a fall in blood glucose during strenuous exercise.

Feeding a fat supplemented diet to 2-year-old Thoroughbreds during training did not change red or white blood cell numbers or liver function as measured by serum levels of AST, GT and SDH. Fat supplemented diets can be fed safely for extended periods of time to horses in training.

This research was published in *Proceedings of the 12th Equine Nutrition and Physiology Society Symposium*, 1991. ✦

A Comparison of Different Standardized Exercise Tests (SET) for Assessing Fitness on a High Speed Treadmill

J.D. Pagan, H.Q. Murphy, and S.G. Jackson

Six Thoroughbred horses were used to evaluate one-day and five-day standardized exercise tests (SET) on a high-speed treadmill with a 6-degree incline. Heart rates were measured and blood samples were collected at rest and during exercise. Heart rate/velocity and lactate/velocity relationships were determined.

This study demonstrated that SET design affects the heart rate and lactate responses to exercise at the same speed. Sequential steps of short duration measure the speed with which aerobic metabolism can increase in response to an increase in exercise intensity, while single steps of longer duration give a truer indication of the overall contribution that anaerobic metabolism makes to energy generation at a specific speed.

This was published in *Proceedings of the 13th Equine Nutrition and Physiology Society Symposium*, 1993.✦

Effect of Chronic Administration of the Oral Antibiotic Sulfamethoxazole and Trimethoprim (SMZ) on Digestibility in Mature Horses

J.D. Pagan and S.G. Jackson

This experiment was designed to test whether chronic administration of sulfamethoxazole and trimethoprim (SMZ) affects digestibility in mature horses.

Four horses were divided into two groups and fed a complete pelleted diet either with or without SMZ for a four-week period. During the last five days of the period, both feed and feces were sampled and analyzed for chemical content.

Chronic SMZ supplementation did not affect the digestibility of any of the nutrients measured in this study. None of the horses showed any signs of digestive upset while receiving the SMZ.

This research was published in *Proceedings of the 13th Equine Nutrition and Physiology Society Symposium*, 1993. ✦

The Effect of Different Fat Sources on Exercise Performance in Thoroughbred Racehorses

J.D. Pagan, W. Tiegs, S.G. Jackson, and H.Q. Murphy

This study was conducted to evaluate the effect of dietary energy source on energy utilization during exercise in nine Thoroughbred racehorses. Two different sources of dietary fat were compared, both alone and as a mixture, to a more traditional high carbohydrate diet during this six-month Latin square design study.

The control diet was a grain-based pelleted diet with oats and corn providing most of the starch. The soy diet was a grain-based pellet with 10% added soybean oil. The coconut diet was a grain-based pellet with 10% added coconut oil. The mixed diet was a grain-based pellet with 5% added soy oil and 5% added coconut oil.

The horses were exercised on a treadmill to determine baseline fitness and ability. They were then placed in four dietary groups for three weeks. During the fourth week, the horses performed standardized exercise tests (SET) on the treadmill over five days, with speed increasing each day. Heart rate was monitored and blood samples were analyzed for glucose, ammonia, and lactic acid. During the fifth week the horses performed a second SET with a longer period at a gallop. Heart rate was recorded and blood samples were taken.

Feeding fat affected the horses' metabolic response to a SET. At the top speed run in this test, plasma lactates averaged 56% higher in the control group vs. the mixed fat treatment. Because of the exponential nature of lactate accumulation with increasing speed, this difference could have a major impact on time to fatigue in racehorses.

This research was published in *Proceedings of the 13th Equine Nutrition and Physiology Society Symposium*, 1993.✦

The Effect of Early Weaning on Growth and Development in Thoroughbred Foals

J.D. Pagan, S.G. Jackson, and R.M. DeGregorio

The purpose of this study was to compare the growth and development of Thoroughbred foals raised under four different management systems.

Forty foals were placed in four treatment groups. Group 1 foals were weaned at 5 days of age and fed a commercial milk replacer, then a milk transition formula pellet, and then a mixture of sweet feed and foal pellets. Foals in group 2 were weaned at 5 months, receiving creep feed shortly before weaning. Foals in group 3 were weaned at 5 months but received creed feeding from 3 months of age. Foals in group 4 were raised on nurse mares, mostly draft and draft cross types.

Foals were weighed and measured every two weeks. Nurse mare foals were heaviest and had the largest heart girths, while milk replacer foals were lightest and had the smallest heart girths at several stages of the study. However, by 168 days the milk replacer foals were as tall as conventionally raised foals and only slightly lighter.

This research was published in *Proceedings of the 13th Equine Nutrition and Physiology Society Symposium,* 1993. ✦

Lactate Threshold and Onset of Blood Lactic Acid Changes During Training Period of Four Months

L. Krusic, A. Usaj, and J.D. Pagan

During a digestion trial, four Standardbred horses were subjected to a standardized exercise test on an 800-meter training track. Running speed and heart rate were determined at lactate threshold (LT) and onset of blood lactate accumulation (OBLA = 4 mmol/l). During four months of endurance training, running speed and heart rate corresponding to LT and OBLA were not improved in a similar manner as expected. Therefore, training may have caused different effects on both exercise intensities.

Properly designed endurance training should include the continuous monitoring of both parameters (speed and heart rate at LT and OBLA). The results of this study suggest that it is important to carry out the standardized exercise test to produce an initial HR of no more than 120 b/min. To obtain an adequate lactate curve, at least five or more running speeds are recommended.

This research was published in *Proceedings of the 13th Equine Nutrition and Physiology Society Symposium*, 1993. ✦

Nutrient Digestibility in Horses

J.D. Pagan

Kentucky Equine Research has conducted dozens of digestibility trials to evaluate how well various nutrients are absorbed from different types of feeds and feed ingredients. For each trial, a standardized experimental design was used to measure digestibility. Horses were fed different diets for three weeks followed by a five-day complete fecal collection period. Samples of feed and feces were analyzed for nutrient content. By comparing the analyses, digestibility of each nutrient was calculated.

Using this pattern, 30 different diets have been evaluated. They have ranged from alfalfa hay to a combination of sweet feed and fescue hay to pelleted concentrates fed with timothy hay. The results have yielded information on nutrient requirements and interactions as well as energy, protein, fiber, carbohydrate, fat, and mineral content of a number of horse feed types.

This research was conducted in 1994 and published in *Advances in Equine Nutrition*. ✦

The Effect of Chromium Supplementation on Metabolic Response to Exercise in Thoroughbred Horses

J.D. Pagan, T. Rotmensen, and S.G. Jackson

Six trained Thoroughbred horses were used in a two-period switch-back design experiment to evaluate the effect that chromium supplementation has on metabolic response to a standardized exercise test (SET) on a high-speed treadmill.

During each 14-day period, the horses were divided into two groups and fed 3.64 kg of a textured grain mix, 2.73 kg of an alfalfa/oat plant forage cube, and 3.64 kg of orchard grass hay. One group (3 horses, CHROM) also received 5 mg of chromium from a chromium yeast product. For the first 12 days of each period, the horses exercised 3 days per week (1 mile at a 4 m/s trot and 1 mile at 7-10 m/s) on a high-speed treadmill. At the end of each period, the horses performed an SET on the treadmill 3 hours after eating 1.82 kg of grain. The SET consisted of a 2 min warm-up walk followed by an 800 m trot (around 4 m/sec), then 800 m gallops of 8 m/s, 9 m/s, 10 m/s, and 11 m/s. These gallops were followed by an 800 meter warm-down trot and 2 min walk. At the end of each speed, heart rate was measured and a blood sample was taken. Blood samples were also taken at 15 and 30 minutes after exercise. After period 1, the diets were switched and the protocol was repeated.

Blood insulin was lower in the CHROM group 1 hr after grain feeding. Blood glucose was significantly lower in the CHROM group after the 8 m/s and 9 m/s steps. Plasma lactate was lower in the CHROM group after the 11 m/s step. Cortisol was significantly lower in the CHROM group 3 hours after feeding, after the warm-up trot, and after the 8 and 11 m/s steps. Heart rate was unaffected by diet. These data suggest that 5 mg of daily supplemental chromium as chromium yeast affects metabolic response to a SET in fit Thoroughbred horses. These responses included reduced lactate and cortisol production which would be beneficial for strenuously exercised horses.

This research was published in *Proceedings of the 14th Equine Nutrition and Physiology Society Symposium*, 1995. ✦

The Effect of Pre-Exercise B-Vitamin Supplementation on Metabolic Response to Exercise in Thoroughbred Horses

T. Rotmensen, J.D. Pagan, and S.G. Jackson

Performance horses are often administered large quantities of B-vitamins before exercise in an attempt to improve performance. B-vitamins play an important role in the conversion of pyruvate into acetyl Co-A for use in aerobic energy generation. A reduction in the conversion of pyruvate to acetyl Co-A will result in lactate production which may contribute to fatigue during exercise.

To evaluate whether B-vitamin supplementation before exercise can affect performance, a 2 X 2 Latin square experiment was conducted. During the first of two periods, four trained 3 year old Thoroughbreds (3 geldings and 1 filly) were divided into 2 groups and fed either a sweet feed and hay diet (CONTROL) or the control diet plus extra B-vitamins the evening before and morning of a standardized exercise test (SET) on a high speed treadmill. The B-vitamin supplement provided 500 mg thiamine, 35 mg riboflavin, 35 mg niacinamide, and 450 mg of d-pantothenic acid per dose. This was added to the evening feed the night before the SET and in the morning feed which was fed 3 hours before the SET. The sweet feed used in this experiment was also fortified with B vitamins so that both groups received daily B-vitamin supplementation throughout the experimental period.

The SET consisted of a 2 min warm-up walk followed by an 800 m trot (about 4 m/s), then 800 m gallops of 8 m/s, 9 m/s, 10 m/s, and 11 m/s. These gallops were followed by an 800 meter warm-down trot and 2 min walk. At the end of each speed, heart rate was measured and a blood sample was taken. Blood samples were also taken at 15 and 30 minutes after exercise. After period 1, the diets were switched and protocol repeated. The lactate-velocity and heart rate-velocity relationships were determined.

There was no statistical difference ($p<.10$) for either lactate level or oxygen use between control and B-vitamin treatment. There was also no difference in plasma glucose either during or after the SET. Additional B-vitamin supplementation before exercise did not appear to affect metabolic response in horses already receiving daily B-vitamin supplementation. It remains to be seen whether this type of B-vitamin supplementation will affect exercise response in horses receiving no supplemental vitamins.

This research was conducted in 1995 and published in *Advances in Equine Nutrition*. ✦

The Influence of Time of Feeding on Exercise Response in Thoroughbreds Fed a Fat Supplemented or High Carbohydrate Diet

J.D. Pagan, I. Burger, and S.G. Jackson

Six Thoroughbred horses were used in a two-period switchback design study to evaluate the influence of time of feeding on exercise response in horses receiving either a traditional sweet feed or a diet supplemented with fat. The horses were placed in two groups and were fed sweet feed or sweet feed with 340 g of soybean oil per day. After eight months on these diets, the horses performed a standardized exercise test on an inclined treadmill. The tests were performed after an overnight fast or at 3 or 8 hours after eating. Heart rate was measured and blood samples were taken before, during, and after exercise and analyzed for glucose, lactate, and insulin.

Heart rate, glucose, and insulin were increased at the beginning of exercise when the horses were fed 3 hours before exercise. Fat-supplemented horses had lower heart rates and insulin levels. Lactate was unaffected by time of feeding or diet. Horses fed three hours before exercise experienced large drops in blood glucose during exercise. Fat supplementation did not affect blood glucose during exercise.

This research was published in *Proceedings of the 14th Equine Nutrition and Physiology Society Symposium*, 1995. ✦

Responses of Blood Glucose, Lactate and Insulin in Horses Fed Equal Amounts of Grain With or Without Added Soybean Oil

J.D. Pagan, T. Rotmensen, and S.G. Jackson

Several studies have shown that when fat is substituted for carbohydrate in equine diets with the same caloric level, blood glucose and insulin responses to feeding are reduced. These studies, however, haven't shown if this response is simply due to reduced glucose in the diet or if fat affects glycemic response. Therefore, this experiment was designed to evaluate whether adding fat to a grain meal would affect glucose and insulin responses to feeding when the level of grain intake remained the same.

Nine horses were used in a two-period switch-back design experiment. Five of the horses were in training and were physically fit and four horses were untrained. During period one, each horse was fed 2.27 kg of a grain mix which consisted of 72% oats, 20% corn, and 8% molasses at 7:00 AM. Five of the horses were also fed 200 ml (170 g) of soybean oil mixed into the grain. At 8:00 AM each horse was given 2.72 kg of mature bluegrass hay. Blood samples were taken from each horse by jugular catheter before feeding, and at 1, 2, 3, 4, 6, 8, 10, and 12 hours after feeding. Water was available to the horses at all times. Blood plasma was analyzed for glucose, insulin, and L-lactate. The same procedure was followed two weeks later with the soybean oil added to the grain of the four horses that served as controls during period 1.

Blood glucose was significantly lower one hour after feeding when soybean oil was added to the diet. Glucose remained lower for 3 hours post feeding. After 6 and 10 hours, blood glucose was higher in the fat-supplemented group. Insulin was lower in the fat-supplemented group 1 hour after feeding. After 8 and 10 hours, insulin was higher in the fat supplemented group. Plasma L-lactate tended to be higher in the control group 4 hours after feeding and higher in the fat supplemented group 6 hours after feeding.

These data suggest that the addition of fat (soybean oil) to a grain meal will affect glucose and insulin response to feeding. These effects are independent of the amount of carbohydrate in the diet and may be due to differences in the rate of gastric emptying when fat is included in the diet.

This research was published in *Proceedings of the 14th Equine Nutrition and Physiology Society Symposium*, 1995.✦

The Incidence of Developmental Orthopedic Disease on a Kentucky Thoroughbred Farm

J.D. Pagan and S.G. Jackson

The incidence of developmental orthopedic disease (DOD) on a commercial Thoroughbred farm was studied over a four-year period. A total of 271 foals were monitored.

DOD was diagnosed in 10% of the foals. Fetlock osteochondritis dissecans (OCD) tended to occur before 180 days of age while hock, shoulder, and stifle OCD occurred around 300-350 days of age. Foals that developed hock and stifle OCD as yearlings tended to be large foals at birth that grew rapidly from 3 to 8 months. These foals were heavier than the average population as weanlings. Foals that developed fetlock OCD before 6 months of age were born early in the year (January, February, or March).

The results of this study suggest that growth rate and management may affect the incidence of certain types of DOD.

This research was published in *Pferdeheilkunde*, 1996. ✦

A Summary of Growth Rates of Thoroughbreds in Kentucky

J.D. Pagan, S.G. Jackson, and S. Caddel

Over a three-year period (1993-1995), a total of 350 Thoroughbred colts and 350 Thoroughbred fillies in central Kentucky were weighed monthly on a portable electronic scale through 18 months of age. Wither height and condition score were also measured in about half of the foals. In order to estimate mature body size in these horses, 472 broodmares were weighed 60 to 90 days after foaling. In addition 25 Thoroughbred breeding stallions were also weighed.

The average body weight of the broodmares equaled 570 kg and the average weight of the stallions equaled 580 kg. At 14 days of age, colts and fillies weighed an average of 77.7 and 76.1 kg and had average heights of 107.3 and 106.3 cm, respectively. Colts were heavier and taller than fillies throughout the study and at 490 days averaged 9.9 kg heavier and 1.6 cm taller. The greatest difference in condition score between sexes occurred at four months of age when the fillies had an average score of 6.48 and the colts had a score of 6.0.

Compared to March foals, foals born in January and February were 6.8 kg lighter at 14 days of age. They remained smaller until about 9 months of age when they averaged about the same as the March foals. April and May foals were larger at 14 days of age than March foals, and remained slightly heavier until 6 months of age.

Average daily gain (ADG) among the four groups was similar until about 7 months of age. ADG was 1.5-1.7 kg/d during the first month and declined linearly to about .70-.80 kg/d at 7 months of age. After 7 months of age, ADG tended to be more variable and by 12 months, ADG was very different between the different months of birth. Foal growth rates were reduced during the winter months regardless of when the foals were born and increased during April and May of the foal's yearling year. Growth rate in these yearlings was more a function of season of the year than age.

This research was published in *Pferdeheilkunde*, 1996. ✦

The Effect of an All-Forage Diet (Alfamaize) or a High-Grain Diet on Metabolic Response to Exercise in Thoroughbred Horses

J.D. Pagan

Carbohydrates are the first source of energy for exercising horses. As exercise periods lengthen, horses begin to use more free fatty acids. Availability of free fatty acids is suppressed by the elevated insulin levels resulting from a high-starch meal before exercise. However, fasting may negatively affect performance by decreasing carbohydrate availability. Feeding the performance horse a high-quality forage may eliminate the problems experienced with either high grain intakes or overnight fasts. Since forage doesn't cause large peaks in blood insulin, forage-fed horses should be able to maintain steady blood glucose levels during exercise. In addition, forage fed the night before and morning of an event should provide continual gluconeogenic substrate in the form of propionic acid to maintain adequate liver glycogen stores.

This study was designed to evaluate how an all-forage diet would affect metabolic response to a strenuous standardized exercise test on a high-speed treadmill.

Results showed that high-quality forage cubes can be fed as the sole source of energy to horses during moderate intensity training. There were no apparent detrimental effects from the type of diet, and horses on this diet performed a standardized exercise test as well as when they were eating a grain/hay diet.

This research was conducted in 1996. ✦

Effect of Supplemental Energy Intake on Growth Rate of Suckling Quarter Horse Foals

L. H. Beuer, R.A. Zimmerman, and J.D. Pagan

Studies of mares and foals maintained in dry-lot conditions indicated that foals need to be supplemented with 15-20 MJ of digestible energy per day at 2 to 3 months of age to obtain weight gains acceptable in commercial horse operations. Acceptable rates of gain were obtained in 3- to 6-month-old foals receiving 27 MJ of digestible energy per day of supplemental energy while maintained on pasture with their dams. Calculations indicate that these foals were likely consuming pasture dry matter at a rate of at least 1% of body weight.

Another study showed that no supplemental energy was needed in foals up to 4 months of age when mares and foals were maintained on high quality pasture.

This was published in *Pferdeheilkunde,* 1996. ✦

The Effect of Exercise on Digestibility of All Forage or Mixed Diet in Thoroughbred Horses

J.D. Pagan, P. Harris, T. Brewster-Barnes, S.E. Duren, and S.G. Jackson

Four conditioned Thoroughbred geldings were used in a 2 x 2 factorial design to investigate the effect of exercise and diet on apparent nutrient digestibility.

The experiment consisted of 4 periods that were 4 weeks in length. During each period, the horses were fed either an all forage diet (4.54 kg alfalfa cubes + 5.45 kg alfalfa/grass hay) (FORAGE) or a mixture of forage and grain (3.63 kg sweet feed + 2.72 kg alfalfa grass hay + 2.27 kg alfalfa cubes) (MIXED). During each period, one horse from each diet was exercised daily on a high speed treadmill (EX). During the fourth week of each period, a complete collection digestion trial was conducted. Each morning during the collection period, the EX horses performed an exercise bout on the treadmill (inclined to 3°) which consisted of a 5 min warm-up walk, 1600 m at 4 m/s, 1600 m at 7 m/s, 1600 m at 9 m/s, 1600 m at 7 m/s, 1600 m at 4 m/s, and a 5 min warm-down walk. Each afternoon, the EX horses were hand walked 1600 m. The NON-EX horses were hand walked 1600 m twice daily.

The dry matter digestibility of the MIXED diet was significantly higher than the FORAGE diet. ADF, NDF, and hemicellulose digestibility were significantly higher in the FORAGE diet. Exercise resulted in a small but statistically significant decrease in dry matter digestibility. This decrease in DM digestibility was also reduced in the EX horses.

This research was published in *Proceedings of the 15th Equine Nutrition and Physiology Society Symposium*, 1997. ✦

Investigation of Time of Hay Feeding on Plasma Volume and Exercise Response in Thoroughbred Horses

J.D. Pagan and P. Harris

Four mature Thoroughbred horses (2 mares and 2 geldings) were used in a 4 x 4 Latin square design experiment to determine whether method of feeding prior to a standardized exercise test (SET) will affect plasma volume and metabolic response to exercise. The four dietary treatments included FAST (12-hr fast prior to SET), AD LIB HAY + GRAIN (ad lib hay from 6 pm the night before and up to SET and 5 lb of a sweet feed mix 2 h prior to exercise), HAY + GRAIN (5 lb of hay fed 3 h prior to SET and 5 lb of grain fed 2 h prior to exercise), and GRAIN (5 lb of hay fed at 10 pm the night prior to SET and 5 lb of grain fed 2 h prior to exercise on the morning of the SET).

The SET was performed on an inclined treadmill (3°) and consisted of a 10 min walk, 10 min trot, 2 min gallop, 10 min walk, 10 min trot, 10 min walk, and 8 min canter. Three hours before the SET, the horses were fitted with an indwelling catheter in the jugular vein. Blood samples were taken hourly before exercise and plasma volume was determined immediately before the SET using an indocyanine green clearance method. During the exercise test, blood samples were taken during the last 30 seconds of each step of the test and 15, 30, 60, 120, and 180 min after exercise. The blood samples were analyzed for glucose, lactate, total plasma protein, PCV, Na^+, Cl^-, and K^+.

The horses were significantly heavier at the beginning of the SET after having ad libitum access to hay. Their plasma volumes were also about 9% smaller compared to the other three treatments. Feeding hay and/or grain 3 hours before the SET did not affect plasma volume. Fasted horses had lower blood lactate after the 8 minute canter compared to the other three treatments. Heart rate was significantly different between treatments. During the 2 min gallop, FAST heart rate averaged 191 while AD LIB HAY + GRAIN equaled 206. During the 8 min canter, FAST heart rate averaged 176 while the heart rate for the AD LIB HAY + GRAIN equaled 191. Heart rates for GRAIN and HAY + GRAIN were intermediate, averaging 183 and 189, respectively. The results of this experiment suggest that an overnight fast before an extended bout of exercise may be beneficial.

This research was published in *Proceedings of the 15th Equine Nutrition and Physiology Society Symposium*, 1997. ✦

A Comparison of Grain, Vegetable Oil, and Beet Pulp as Energy Sources for the Exercised Horse

K.G. Crandell, J.D. Pagan, P.A. Harris, and S.E. Duren

This study compared a traditional high-grain diet to diets that provide 15% of the total caloric intake from either vegetable oil (FAT) or beet pulp (FIBER) in performance horses.

Results showed that blood glucose was significantly lower in the FAT horses during the three hours post-feeding as compared to either the CONTROL or FIBER fed horses. Insulin was also significantly lower in the FAT horses both post-feeding and throughout exercise. Cortisol was significantly lower in the FAT horses as compared to the CONTROL horses during exercise. No differences were found in lactate, total protein, hematocrit, or triglycerides during exercise.

Substituting 15% of the digestible energy as vegetable oil had a greater effect on metabolic response to exercise than a 15% substitution of beet pulp. Beet pulp provided adequate energy to maintain weight and horses performed as well as the horses on the high starch diet. Therefore, it is reasonable to substitute 15% of the energy of the diet of a performance horse with a highly fermentable fiber, but it may not have the performance advantages of 15% added oil.

This was published in *Proceedings of the 16th Equine Nutrition and Physiology Society Symposium*, 1999. ✦

Effect of Diet on the Metabolic Response to Exercise in Thoroughbred Horses with Recurrent Exertional Rhabdomyolysis (RER)

J.M. MacLeay, S.J. Valberg, J.D. Pagan, J. Billstrom, and J. Roberts

The purpose of this study was to investigate the effect of diet on changes in serum creatine kinase (CK), plasma lactate, muscle lactate, and muscle glycogen concentrations with near-maximal exercise. The experimental diets were composed of adequate amounts of soluble carbohydrate or fat, or excessive soluble carbohydrate.

Mean creatine kinase (CK) activity post-exercise on the high-carbohydrate diet was 1.5 times greater than the fat and low-carbohydrate diet, but this was not significantly different. The changes in plasma lactate and muscle lactate after exercise were significantly higher on the fat diet than the low-carbohydrate diet. Post-exercise muscle lactates were 1.5 times higher on the fat diet than on the low-carbohydrate or high-carbohydrate diet. No significant differences in muscle glycogen were seen between diets.

This study shows diet has a significant effect on muscle metabolism even when fed for a three-week period. CK activity was lowest in Thoroughbred horses susceptible to RER when fed a diet formulated to meet, but not exceed, daily energy requirements using either soluble carbohydrates or fat. The fat diet resulted in higher muscle lactate concentrations in RER horses, though no associated increase in CK was observed.

This research was published in the *Proceedings of the 5th International Conference on Equine Exercise Physiology*, 1998. ✦

Effect of Feeding Thoroughbreds a High Unsaturated or Saturated Vegetable Oil Supplemented Diet for 6 Months Following a 10-Month Fat Acclimation Diet

P.A. Harris, J.D. Pagan, K.G. Crandell, and N. Davidson

The intent of this study was to investigate the effects of feeding a diet in which approximately 20% of the digestible energy came from supplemental fat (saturated or unsaturated vegetable oil) for six months following a ten-month acclimation period in which supplemental fat provided approximately 13% of the digestible energy.

No significant effect in glucose tolerance test responses for the saturated or unsaturated treatment groups was observed. Apparent total fat digestibilities at the end of the 16-month study for the four horses were 74.8 and 76.5 for saturated plus 79.1 and 71.9% for unsaturated, and these had risen from 58 and 66% for saturated and 58 and 60% for unsaturated during the first week of acclimation. VLa4 and V200 were not significantly affected by either diet during the high fat period. Dry matter, crude protein, and fiber apparent digestibilities were similar for both treatments. No adverse effects of feeding saturated or unsaturated fats on coat condition or hoof appearance were seen.

No adverse effects were identified as a result of feeding a diet supplemented with either a saturated or unsaturated vegetable oil for six months at approximately 22% digestible energy after a ten month acclimation period in which supplemental fat provided approximately 13% of the digestible energy.

This research was published in the *Proceedings of the 5th International Conference on Equine Exercise Physiology*, 1998. ✦

Effects of Different Quantities of Sweet Feed and Corn Oil on Glycemic Response in Thoroughbred Horses

J.D. Pagan, P.A. Harris, M.A.P. Kennedy, N. Davidson, and K.E. Hoekstra

The addition of fat to a grain meal causes a reduction in both glucose area under the curve and peak glucose values. This study was conducted to determine if the reduction in glycemic response is proportional to the quantity of fat added to a grain meal, while maintaining the isocaloric value of the treatment diets.

Six Thoroughbred horses were used in a 3 x 6 Latin square design. The horses were fed mixed grass hay and one of three diets: sweet feed, sweet feed plus 75 g corn oil, or sweet feed plus 150 g corn oil. After an adaptation period, blood samples were taken before and at 30-minute intervals after feeding and analyzed for glucose values. Plasma glucose variables were statistically analyzed by the general linear model procedure for the analysis of variance. The effect of corn oil on glycemic index was also assessed by taking glucose area under the curve for the sweet feed only treatment diet as the standard of reference.

Results indicated no statistically significant differences between dietary treatments for area under the curve, mean glucose, peak glucose, or time to peak glucose. However, as in previous studies, addition of corn oil to a meal of sweet feed was associated with reductions in area under the glucose vs. time curve and peak glucose concentrations, and the glycemic indices for the low-oil and high-oil diets were lower when compared to a meal of sweet feed alone.

This research was conducted in 1998. ✦

Exercise Affects Digestibility and Rate of Passage of All-Forage and Mixed Diets in Thoroughbred Horses

J.D. Pagan, P. Harris, T. Brewster-Barnes, S.E. Duren, and S.G. Jackson

The purpose of this study was to measure the effect of 8 km daily trotting and galloping exercise on the digestibility and rate of passage of either an all-forage or a mixed forage/grain ration in trained Thoroughbred horses.

Dry matter and nonstructural carbohydrate digestibility of the MIXED diet were significantly higher than those of the FORAGE diet. ADF, NDF, and hemicellulose were significantly higher in the FORAGE diet. Exercise resulted in a small but significant decrease in dry matter digestibility. This decrease in DM digestibility was primarily from a reduction in ADF digestibility. Potassium digestibility was also significantly reduced in exercised horses, and sodium was increased.

Rate of passage in the FORAGE diet was faster than the MIXED diet, probably due to a combination of increased saliva flow and greater water intake. Exercise reduced mean retention time. Exercised horses consumed more water than unexercised horses, suggesting that water intake may affect rate of passage.

A decrease in dry matter digestibility occurred when horses were exercised. Although this reduction in dry matter digestibility is statistically significant, its practical significance is questionable. Of more interest are the large differences in rate of passage between the FORAGE and MIXED diets. More research is required to determine what factors affect rate of passage in horses and how these differences affect feed utilization and performance.

This research was published in *Journal of Nutrition*, 1998. ✦

Time of Feeding and Fat Supplementation Affect Exercise Response in Thoroughbred Horses

S.E. Duren, J.D. Pagan, P.A. Harris, and K.G. Crandell

The objectives of this study were to further define the impact of feeding prior to exercise and to determine if replacing a portion of the carbohydrate from a typical performance horse diet with fat would alter hormone and substrate concentrations during exercise.

Time of feeding influenced plasma glucose concentrations during exercise. Blood glucose dropped in the horses fed three hours before exercise and was significantly lower during and after the 11 m/s gallop. Fat supplementation affected plasma glucose concentrations post-exercise. Plasma glucose concentrations were higher post-exercise for fat-supplemented horses compared with control horses. Horses that were fed three hours prior to exercise tended to have higher heart rates compared to the 8-hour postprandial and fasted horses. During the warm-up walk, fat-supplemented horses had lower heart rates. Plasma lactate increased with exercise intensity but was not affected by dietary treatment. Time of feeding did not influence lactate concentration during exercise. Insulin was significantly higher in the 3-hour fed horses at the beginning of and throughout exercise. Insulin was lower in the fat supplemented horses following the warm-up and during exercise.

Results from this study and from other studies that have investigated pre-exercise feeding indicate that time of feeding has an influence on circulating blood metabolites available for use during exercise. Providing a textured feed 3 hours prior to exercise may be beneficial but is subject to interpretation. On one hand, starting exercise with high plasma glucose concentration may be viewed as having a readily available energy source. However, the potential of hyperinsulinemia to negatively influence plasma concentrations of nutrients and hormones appears to outweigh the potential benefit of feeding horses three hours prior to exercise.

This research was published in the *Proceedings of the 5th International Conference on Equine Exercise Physiology*, 1998. ✦

Timing and Amount of Forage and Grain Affect Exercise Response in Thoroughbred Horses

J.D. Pagan and P.A. Harris

Three experiments were conducted to evaluate if feeding hay with and without grain affects glycemic and hematological responses in Thoroughbred horses at rest and during a simulated competition exercise test on a high speed treadmill. The first experiment evaluated how feeding forage along with grain influences plasma variables and water intake. The second experiment was conducted to determine whether these changes affect exercise performance. The third experiment was conducted to determine how forage alone affects exercise response.

Results showed that time of hay feeding affects glycemic response, plasma protein, and water intake post grain feeding. Free-choice hay feeding resulted in a 9% reduction in plasma volume. Fasted horses had lower blood lactate after exercise compared to the grain fed treatments. Heart rate was significantly different between treatments. Feeding only forage before exercise had a much smaller effect on glycemic and insulin response to exercise than a grain meal. Forage did not affect free fatty acid availability.

This study showed that grain should not be fed immediately before exercise. Small amounts of hay or grazing do not adversely affect performance and will stimulate saliva production which may help preserve gastrointestinal integrity.

This research was published in *Proceedings of the 5th International Conference on Equine Exercise Physiology*, 1998. ✦

Voluntary Intake of Loose Versus Block Salt and Its Effect on Water Intake in Mature Idle Thoroughbred Horses

M.A.P. Kennedy, P. Entrekin, P. Harris, and J.D. Pagan

The purpose of this study was to measure the voluntary intake of loose versus block salt over time and evaluate how salt intake affects water consumption.

Four mature Thoroughbred horses were used in the eight-week switch-back design. Daily water consumption was measured, and salt intake from either loose or block sources was measured weekly.

Horses with free-choice access to loose salt drank significantly more water than when offered block salt. Salt intake was more consistent from week to week when offered in block form.

This research was conducted in 1998 and published in *Advances in Equine Nutrition II*. ✦

The Influence of Fruit Flavors on Feed Preference in Thoroughbred Horses

M.A.P. Kennedy, T. Currier, J. Glowaky, and J.D. Pagan

This study was conducted to evaluate if rate of intake of oats could be influenced by the addition of fruit flavors.

Eight mature Thoroughbred geldings were used in a replicated 4 x 4 Latin square two-choice preference test using apple, cherry, teaberry, and citrus flavors in a carrier of wheat midds. Two horses per period were offered each treatment along with a control (no flavoring). After five minutes, the amount of oats remaining in each bucket was measured.

There was a trend toward consumption of the flavored oats as compared to the control. Cherry was the most favored flavor. There was little difference in appeal of the other three flavors.

This research was conducted in 1998 and published in *Advances in Equine Nutrition II*. ✦

The Effects of Diet and Exercise on the Behavior of Stabled Horses

N. Davidson, P. Harris, D. Goodwin, S. Cook, and J.D. Pagan

In this study, stabled horses were managed on all combinations of two exercise regimens (light or strenuous) and two diets (forage or forage/grain). Observations of behavior were made on three consecutive days in the second and fourth week of each four-week treatment period. Behavior was noted while the horses were in stalls and also as they were being handled in a set routine (grooming, application of fly spray, fitting of a heart monitor with surcingle).

Horses that had been lightly exercised showed more uncooperative behavior during handling. Restless behavior in the stall was more pronounced in horses on the mixed diet. There was an interaction between diet and exercise for a number of redirected oral behavior patterns such as licking objects.

This research was published in *Proceedings of the 32nd International Congress of the International Society of Applied Ethology*, 1998. ✦

Carbohydrate Supplementation of Horses During Endurance Exercise: Comparison of Fructose and Glucose

S.R. Bullimore, J.D. Pagan, P.A. Harris, K.E. Hoekstra, K.A. Roose, S.G. Gardner, and R.J. Geor

To delay the onset of fatigue, endurance horses are often fed at rest stops during races. The resulting increase in blood insulin may adversely inhibit lipolysis. Ingestion of fructose produces a smaller insulin rise than glucose. This study compared glucose and fructose as carbohydrate supplements for endurance horses.

Three Arabian geldings were given 300 g of fructose (F), glucose (G), or 50% glucose/50% fructose (GF), in 1.5-liter water, by stomach tube. In the resting test, carbohydrate was administered at rest. Following treatment, blood samples were taken every 30 minutes for eight hours, and feces were collected for 24 hours.

Treatment did not affect fecal weight or water content. Plasma glucose and insulin responses did not differ among treatments. Post-treatment (60 min) plasma L-lactate tended to be higher after the F and GF treatments than after the G treatment. In the exercise test, two treadmill exercise bouts at 0° incline (bout 1: 90 min; bout 2: 120 min) were separated by a one-hour rest period. A total distance of 36.84 km was covered at a mean speed of 2.9 m/s. Carbohydrate was administered 45 minutes before bout 2. Plasma glucose and insulin at the start of bout 2 were higher with the GF treatment than with the F treatment. However, during exercise, plasma glucose concentrations did not differ among treatments. Fructose is well absorbed by horses and rapidly converted to glucose.

This research was published in *Journal of Nutrition*, 1999. ✦

Effect of Corn Processing on Glycemic Response in Horses

K.E. Hoekstra, K. Newman, M.A.P. Kennedy, and J.D. Pagan

This study was conducted to evaluate how cracking, grinding, or steam processing affects starch digestibility of corn, using glycemic response as an indirect measure of prececal starch digestibility.

The glycemic response of each grain was compared using a glycemic index where each feed's glucose area under the curve was expressed relative to cracked corn. Steam-flaked corn produced a greater glycemic response than cracked or ground corn. Peak glucose was also greater for steam-flaked corn. Horses on the cracked corn diet demonstrated greater peak glucose than those on ground corn. Plasma glucose concentrations were consistently lower for cracked and ground corn treatments, when compared with steam-flaked corn from 90 to 180 minutes post feeding during sample collection. Time to peak glucose was unaffected by processing. Mean lactate, peak lactate, and time to peak lactate were unaffected by processing.

Steam flaking alters glycemic response to a much greater extent than grinding or cracking. Higher peak concentrations of glucose in the plasma of horses fed steam-flaked corn indicated that steam-flaking results primarily in small intestinal digestion, avoiding excessive microbial fermentation in the large intestine and greater lactate production by the hindgut. Further research is necessary to determine if other processing techniques that involve heating the corn kernel, such as extruding or micronizing, will result in similar improvements in glycemic response.

This research was published in *Proceedings of the 16th Equine Nutrition and Physiology Society Symposium,* 1999. ✦

Effect of Selenium Source on Selenium Digestibility and Retention in Exercised Thoroughbreds

J.D. Pagan, P. Karnezos, M.A.P. Kennedy, T. Currier, and K.E. Hoekstra

This study was conducted to evaluate how exercised Thoroughbreds digest and retain selenium from either sodium selenite or Se-enriched yeast-supplemented diets.

The apparent absorption of SELENITE and Se-YEAST selenium averaged 51.1% and 57.3% respectively. Most of the difference in selenium retention was the result of increased selenium absorption since there was no difference in average daily urinary Se excretion between the two supplemental sources. Following exercise, Se excretion in the SELENITE group was significantly higher than during non-exercise days of the collection period. A similar increase did not occur in the Se-YEAST group. Urinary Se excretion was lower in the Se-YEAST group compared to the SELENITE fed horses on the day of exercise. Both plasma and whole blood Se increased post-exercise. Red blood cell Se was similar between both treatment groups and there was a trend towards a decrease in red blood cell Se post-exercise. Plasma Se remained elevated in both treatments 4 hours post-exercise. By 24 hours post-exercise, the SELENITE Se had returned to pre-exercise levels, while plasma Se from the Se-YEAST-supplemented group remained elevated. At this time, Se-YEAST plasma Se was higher than the SELENITE group.

Increased urinary Se excretion following exercise in the SELENITE group suggests that the requirement for Se by exercised horses may be dependent on Se source and exercise frequency. More research is needed to quantify the Se requirement of horses at different exercise intensities and to determine how the form of dietary Se affects antioxidant status.

This research was published in *Proceedings of the 16th Equine Nutrition and Physiology Society Symposium*, 1999. ✦

An Evaluation of Corn Oil, Rice Bran, and Refined Dry Fat as Energy Sources for Exercised Thoroughbreds

M.A.P. Kennedy, J.D. Pagan, K.E. Hoekstra, E. Langfoss, and K. Heiderscheidt

This study was conducted to compare the digestibility of several fat sources (dry fat, rice bran, corn oil) and evaluate how well they functioned as energy sources for horses during exercise.

Apparent fat digestibility was similar between all three supplemental fat sources and significantly higher than the control treatment. The estimated fat digestibility of each fat source was very high (88-94%) and not significantly different. There was little problem with palatability with any of the fat sources. Lactate was significantly lower in the rice bran treatment compared to the corn oil treatment post-exercise. Lactates in the control and dry fat treatments were similar throughout exercise. Heart rates during the gallop were significantly lower in the control and rice bran horses compared to the dry fat and corn oil treatments. There was also a trend for heart rate to be lower in the rice bran treatment than in the corn oil treatment during the 8-minute canter.

The results of this study suggest that fat from corn oil, rice bran and refined dry fat can be used effectively in rations for exercising horses. The vegetable oil fats appeared to affect triglyceride mobilization and/or clearance and this was probably due to a change in lipoprotein lipase activity in the adipose tissue and muscle. Feeding rice bran resulted in lower lactate accumulation and lower heart rates during exercise compared to corn oil. More research is needed to determine the reason for these differences.

This research was published in *Proceedings of the 16th Equine Nutrition and Physiology Society Symposium*, 1999. ✦

Feed Type and Intake Affect Glycemic Response in Thoroughbred Horses

J.D. Pagan, P.A. Harris, M.A.P. Kennedy, N. Davidson, and K.E. Hoekstra

The purpose of this experiment was to determine glycemic response in horses fed six different feeds (sweet feed, whole oats, cracked corn, high fiber mix, sweet feed plus oil, alfalfa forage) at three different levels of intake (1.65, 3.31, or 5.51 lb).

Area under the curve indicated differences in glycemic response between low (1.65 lb) and high (5.51 lb) intake levels of all diets combined. Sweet feed and whole oats demonstrated the greatest glycemic response, while alfalfa and sweet feed plus corn oil provided the lowest response. Plotting the glycemic index by feed and level of intake revealed an appreciable drop in the index for whole oats fed at 5.51 lb compared to that at 1.65 lb and relative to glycemic indexes generated for other feeds. Mean glucose was highest for sweet feed, whole oats, and the low starch/high fiber mix and lowest for the alfalfa diet. Peak glucose was similar for all diets except alfalfa forage. Time to peak glucose was greatly increased in the sweet feed plus corn oil diet, while the remaining diets demonstrated similar responses. Increasing level of intake from 1.65 lb to 3.31 lb per feeding increased time to peak glucose by 45 ± 14.1 minutes.

Results of this study indicate that different grain diets demonstrate different glycemic responses and adding fat reduces both the area under the curve and peak glucose values, as measured within this experimental design. More research is required to determine the relevance of glycemic response in predicting the effects of different feed ingredients on a horse's performance or behavior.

This research was published in *Proceedings of the 16th Equine Nutrition and Physiology Society Symposium*, 1999. ✦

Time of Feeding and Fat Supplementation Affect Plasma Concentrations of Insulin and Metabolites During Exercise

S.E. Duren, J.D. Pagan, P.A. Harris, and K.G. Crandell

Six Thoroughbreds were used to evaluate time of feeding on changes in exercise response in horses receiving either a textured feed or a fat-supplemented textured feed.

Using a crossover design, 3 horses were fed a fat-supplemented diet while 3 horses received a control ration of textured feed. Horses performed a standardized exercise test (SET) on a high speed treadmill. The SET was performed at 3 different times: 1) following an overnight 12 h fast, 2) 3 h after feeding and 3) 8 h after feeding. The SET consisted of a 2 min walk at 1.4 m/s, 800 m trot at 4.2 m/s, 800 m gallop at 7.7 m/s, 1600 m gallop at 11 m/s, 800 m trot at 4.2 m/s and 2 min walk at 1.4 m/s. Jugular blood samples were taken before feeding, hourly until the beginning of the SET, at the end of each exercise step, 15 min post exercise and 30 min post exercise. During the SET, heart rate was measured and blood samples collected for analysis of glucose, lactate, insulin and nonesterified fatty acids (NEFA).

Feeding horses 3 h prior to exercise resulted in elevated concentrations of plasma glucose and insulin ($P < 0.01$) at rest. Elevated concentrations of insulin in horses fed 3 h prior to exercise decreased plasma glucose ($P < 0.01$) during exercise and appeared to have suppressed fat oxidation during exercise because horses that were either fasted or fed 8 h post prandial had a net disappearance of NEFA in the plasma during exercise. This study indicates that beginning exercise with elevated plasma insulin appeared to be of no benefit during the exercise conducted in this experiment.

This research was published in *Equine Veterinary Journal*, 1999. ✦

Time of Feeding Critical for Performance

J.D. Pagan

One of the most frequently asked questions regarding feeding the performance horse is when to feed before a competition. Several studies have evaluated how feeding grain before exercise affects plasma concentrations of nutrients and hormones and substrate utilization during exercise. In each of these studies, a pre-exercise concentrate meal suppressed free fatty acid (FFA) availability and enhanced glucose uptake by muscle during exercise. Forage was not fed with the concentrate in any of these studies. Thus, it is not known whether feeding hay along with grain will alter substrate availability during exercise.

Therefore, a series of experiments was conducted to first evaluate how feeding forage along with grain influences plasma variables and water intake and then to determine whether these changes affect exercise performance. Additionally, a study was conducted to determine how forage alone affects exercise response. Since time of feeding is particularly important for three-day event horses, the exercise test used was a competition exercise test (CET) performed on a high speed treadmill and designed to simulate the physiological and metabolic stresses of the speed and endurance test of a three-day event.

Feeding only forage before competition does not appear to interfere with FFA availability, and has no adverse effects other than possibly reducing plasma volume and increasing body weight. Feeding grain 2 h before exercise will reduce FFA availability and increase glucose uptake by the working muscle.

This research was conducted in 1999 and published in *Advances in Equine Nutrition II*. ✦

Effect of Ration and Exercise on Plasma Creatine Kinase Activity and Lactate Concentration in Thoroughbred Horses with Recurrent Exertional Rhabdomyolysis

J.M. MacLeay, S.J. Valberg, J.D. Pagan, J.L. Xue, F.D. De La Corte, and J. Roberts

This study was designed to determine the effects of three rations (low grain, fat, high grain) on plasma creatine kinase (CK) activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis (RER).

The study used five Thoroughbreds with RER and three healthy Thoroughbreds (control horses). Rations were formulated to meet (low-grain and fat rations) or exceed (high-grain ration) daily energy requirements. Each ration was fed to horses in a crossover design for three weeks. Horses were exercised on a treadmill Monday through Friday; maximum speed on Monday and Friday was 11 m/s (6% slope), on Tuesday and Thursday was 9 m/s, and on Wednesday was 4.5 m/s. Plasma CK activity and lactate concentration were determined before and after exercise.

Horses with RER fed the high-grain ration had significantly greater CK activity and change in CK activity 4 hours after exercise compared with those fed the low-grain ration. Horses with RER exercised at the trot or canter had significantly greater increases in CK activity compared with those exercised at the gallop. Plasma lactate concentrations after exercise were similar in control and affected horses. Lactate concentration and CK activity were not correlated in horses with RER. Rations high in grain and formulated to exceed daily energy requirements may increase episodes of rhabdomyolysis in Thoroughbred horses susceptible to RER.

This research was published in *American Journal of Veterinary Research*, 2000. ✦

Effect of an Aluminum Supplement on Nutrient Digestibility and Mineral Metabolism in Thoroughbred Horses

K.A. Roose, K.E. Hoekstra, J.D. Pagan, and R.J. Geor

The aim of this study was to ascertain the effect of an aluminum-containing supplement on nutrient digestibility and mineral balance in Thoroughbreds.

Digestibility was measured with and without aluminum supplementation for calcium, phosphorus, magnesium, potassium, sodium, chloride, iron, zinc, copper, manganese, and aluminum.

Supplementation with aluminum caused no deleterious effects on nutrient digestibility or metabolism of calcium, phosphorus, magnesium, zinc, copper, and boron. A slight drop in digestibility was seen in potassium, chloride, and manganese. The results of this study showed that short-term consumption of a diet containing moderately high levels of aluminum had negligible effect on nutrient digestibility and external mineral balance in mature horses.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001. ✦

Effects of Exercise Training on the Digestibility and Requirements of Copper, Zinc, and Manganese in Thoroughbred Horses

C. Hudson, J.D. Pagan, K. Hoekstra, A. Prince, S. Gardner, and R. Geor

The purpose of this study was to evaluate the effects of exercise on the digestibility and requirements of copper, zinc, and manganese.

Results showed that zinc requirements were significantly higher in exercised horses compared to sedentary horses. There were no differences in requirements for copper and manganese between exercised and sedentary horses. There were trends for lower true digestibility of copper and manganese in exercised horses.

This study suggests that exercise training results in a higher requirement for zinc but does not affect the true digestibility and maintenance requirements of copper and manganese.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001. ✦

Effects of Intake Level on the Digestibility and Retention of Copper, Zinc and Manganese in Sedentary Horses

C.A. Hudson, J.D. Pagan, K.E. Hoekstra, A. Prince, S. Gardner, R.J. Geor

Four mature Thoroughbreds were studied in a 16-week experiment consisting of 4 periods. Each period ended in a 5-day complete collection digestion trial. Horses were fed an unfortified diet in the first period. In the other periods, the diet was fortified with a supplement providing 50, 100, or 200% of requirements for copper, zinc, and manganese. Samples of feed, urine, and feces were collected and analyzed for these minerals, and relationships of intake and retention were determined.

Results showed zinc data agreed with previously published values, but the corresponding values for manganese were 40% higher than previous estimates. The figures for copper retention were in accord with published recommendations.

This research was published in *Advances in Equine Nutrition II.* ✦

Comparison of the Metabolic Responses of Trained Arabian and Thoroughbred Horses During High and Low Intensity Exercise

A. Prince, R. Geor, P. Harris, K. Hoekstra, S. Gardner, C. Hudson, and J.D. Pagan

The study compared the metabolic responses of Arabian and Thoroughbred horses subjected to high- and low-intensity exercise.

Running speed and VO2max (highest rate of oxygen consumable by a horse) were higher in Thoroughbreds than in Arabians. During sprint exercise, Thoroughbreds were able to run longer before fatigue than Arabians. On the other hand, during low-intensity exercise, plasma fatty acid concentrations were higher and respiratory exchange ratio was lower in Arabians, indicating a greater use of fat for energy. Higher aerobic and anaerobic capacity of the Thoroughbreds likely contributed to their superior performance during high-intensity exercise, whereas the Arabians may be better adapted for endurance exercise as evidenced by the greater use of fat.

Variations in muscle type between Arabians and Thoroughbreds may contribute to the differences in metabolic responses during high- and low-intensity exercise tests. Further studies are necessary to determine how diet affects metabolism in these breeds during exercise.

This research was published in *Equine Veterinary Journal*, 2001. ✦

Corn Oil Affects Gastric Emptying

J.D. Pagan

This study was designed to test the hypothesis that gastric emptying is delayed following ingestion of a grain plus corn oil meal compared to a meal of grain alone.

Four mature Arabian horses were studied in a 2 x 2 factorial design study. Horses were fed either a control diet (hay and sweet feed) or an isocaloric diet in which fat was provided by the addition of corn oil. For assessment of solid-phase gastric emptying, the test meals were labeled with 1 g of 13C-octanoic acid. Blood samples for measurement of plasma glucose concentration and 13C-enrichment were collected to calculate half-dose recover time, time to peak blood 13C-enrichment, and gastric emptying coefficient.

The addition of corn oil to a meal of sweet feed results in a delay in solid-phase gastric emptying, and the effect of oil on gastric emptying is not affected by short-term adaptation to a fat-supplemented diet. In addition, the slowing of gastric emptying may contribute to the blunted glycemic response following a grain meal containing corn oil.

This research was conducted in 2001 and published in *Advances in Equine Nutrition*. ✦

Effects of an External Nasal Strip and Furosemide on Pulmonary Hemorrhage in Thoroughbreds Following High-Intensity Exercise

R.J. Geor, L. Ommundson,
G. Fenton, and J.D. Pagan

The purpose of this study was to examine the effects of an external nasal strip (NS), furosemide (FR), and a combination of the two treatments (NS + FR) on exercise-induced pulmonary hemorrhage (EIPH) in Thoroughbred horses. It was hypothesized that both the NS and FR would attenuate EIPH as assessed by red blood cell count in bronchoalveolar lavage fluid.

In random order, 8 horses completed each of 4 sprint exercise tests on a treadmill: 1) NS; 2) FR (0.5 mg/kg bwt i.v., 4 h pre-exercise); 3) NS + FR; and 4) control (C; no treatment). After a 5 min warm-up (4.5 m/s), horses completed 2 min running at 120% maximum oxygen consumption (Vo_2max) with the treadmill set at 3° incline. Mean ± s.d. running speed was 14.2 ± 0.2 m/s. In the FR and NS + FR trials, horses carried weight equal to that lost as a result of furosemide administration. During exercise at 120%V⊠2max, oxygen consumption (Vo_2) and carbon dioxide production (Vco_2) were measured at 15 s intervals. Plasma lactate concentration was measured in samples collected before exercise, at the end of the sprint and after 5 min cool-down at the trot. Thirty minutes after the run, bronchoalveolar lavage (BAL) was performed and the red cell count in the fluid quantified.

Vo_2 and Vco_2 were significantly lower in NS and NS + FR trials than in the C and FR trials at the end of the sprint exercise protocol. However, plasma lactate concentrations did not differ among treatments. Compared with the C trial (61.1 ± 30.5 × 106 red blood cells/ml BAL fluid), pulmonary hemorrhage was significantly decreased in both the NS and FR trials. EIPH in the NS + FR trial was further diminished compared to the NS trial, but not different from the FR trial.

We conclude that both the external nasal strip and furosemide attenuate pulmonary hemorrhage in Thoroughbred horses during high-speed sprint exercise. The external nasal strip appears to lower the metabolic cost of supramaximal exertion in horses. Given the purported ergogenic effects of furosemide, the external nasal strip is a valuable alternative for the attenuation of EIPH.

This research was published in E*quine Veterinary Journal*, 2001. ✦

Effect of Preparation Method on the Glycemic Response to Ingestion of Beet Pulp in Thoroughbred Horses

L. Groff, J.D. Pagan, K. Hoekstra, S. Gardner, O. Rice, K. Roose, and R. Geor

Previous studies have shown a marked glucose response when beet pulp is fed to horses. To determine whether this response is due to residual simple sugars in beet pulp shreds, a study was conducted to evaluate how different preparations of shredded beet pulp affect glycemic response in Thoroughbreds.

Rinsed beet pulp (beet pulp allowed to soak overnight and then rinsed to remove sugar) produced a lower glycemic response, mean glucose, and peak glucose than other treatments, including hydrated beet pulp (allowed to soak overnight and not rinsed prior to being fed), dry beet pulp with molasses, and whole oats.

Removal of glucose in dried beet pulp lowers glycemic response in horses when compared with hydrated beet pulp and dry beet pulp with molasses. This study confirms that simple sugars in shredded beet pulp contribute to an increase in blood glucose after feeding.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001. ✦

Effects of Restricted Hay Intake on Body Weight and Metabolic Responses to High Intensity Exercise in Thoroughbred Horses

O. Rice, R. Geor, P. Harris, K. Hoekstra, S. Gardner, and J.D. Pagan

The goal of this study was to compare ad libitum and restricted (1% of body weight for a three-day period) hay intake on metabolic responses of Thoroughbreds subjected to high intensity exercise.

Free-choice hay intake averaged 9 kg (20 pounds). Three days of restricted hay intake (10 pounds per day) resulted in a 2% decrease in body weight compared with free choice feeding. During a sprint exercise test, oxygen consumption was higher in horses fed restricted hay. Oxygen deficit and peak plasma lactate were higher during exercise in horses fed ad libitum hay.

The reduction in body weight associated with restricted hay feeding coincided with greater oxygen consumption during exercise and a corresponding decrease in anaerobic energy expenditure and accumulation of blood lactate. As lactate accumulation can contribute to fatigue during high intensity exercise, short-term (3-4 days) restriction of hay intake may be beneficial for racehorses.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001. ✦

Glycemic Response to Rice Bran in Thoroughbred Horses

R. Geor, J.D. Pagan, K. Hoekstra, and D. Nash

The objective of this study was twofold: to measure the starch content of rice bran, whole oats, and corn, and to determine the glycemic response to a meal of rice bran in Thoroughbred horses.

Direct analysis revealed rice bran to be 14% starch and therefore substantially lower than whole oats (50%) and corn (61%). Feeding rice bran caused little change in blood glucose concentrations. Compared to a meal of whole oats, the glycemic index for a meal of rice bran was 47%, a level similar to that for a meal of alfalfa hay.

This study affirms that rice bran causes only slight changes in blood glucose concentrations following ingestion. Therefore, rice bran is a suitable feedstuff for horses that require a low starch diet, such as horses with polysaccharide storage myopathy, a muscle disorder.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001.✦

Influence of High Carbohydrate and High Fiber Diet on Cecum Content of Organic Matter and Volatile Fatty Acids

L. Krusic, J.D. Pagan, J. Lorenz, and A. Pen

Four groups of resting adult horses participated in a ten-week feeding experiment. The diets of the resting group of horses (high fiber or high carbohydrate) were supplemented (or not) with a commercially available dietary yeast culture.

After an adaptation period of six weeks, a four-week feeding trial and a final five-day total collection of urine and feces were conducted. After the last collection day, all animals were slaughtered 5 hours after the last meal and cecum digesta samples were taken for analyses of dry matter, crude protein, crude fiber crude fat, ash, pH, NH_3, NH_3-N, total organic acids, lactate, acetate, propionate, and butyrate.

The dry matter, crude protein, crude fiber, crude fat, and ash content did not differ significantly in all four diet groups. Lower pH values in all four diet groups appeared to be closely related to the substantial amount of soluble carbohydrates in the diets. Mean cecal lactic acid concentrations of high fiber and high carbohydrate diet groups were not significantly different. The tendency toward a higher level of lactate accumulation could be detected only in the high fiber diet group with added yeast. The ammonia concentration of cecum digesta showed a lower tendency in the high fiber diet group without added yeast. However, the differences were not significantly different due to marked individual variations. The concentration of NH_3-N showed similar patterns in all four diet groups, with the lowest concentration in the high fiber diet group without added yeast. The concentration of total VFA in the cecum digesta was not influenced by the different diet groups or by yeast supplementation. The mean concentrations of acetate, propionate, and butyrate were consistently lower than the concentration of total organic acids in all four diet groups. The amount of acetate was found to be higher in both the high fiber diet and high carbohydrate diet group without added yeast, but the acetate concentration in the high carbohydrate diet with added yeast did not show a significant lower value. Similar trends were observed in the concentration of cecal propionate. Both propionate and butyrate concentration of cecum digesta were almost the same in all four diet groups.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001. ✦

The Relationship Between Glycemic Response and the Incidence of OCD in Thoroughbred Weanlings: A Field Study

J.D. Pagan, R.J. Geor, S.E. Caddel, P.B. Pryor, and K.E. Hoekstra

The purpose of this study was to evaluate if there is a relationship between a glycemic response test and the incidence of osteochodritis dissecans (OCD) in Thoroughbred weanlings, and to determine if this test would be useful in identifying factors that may predispose young growing horses to OCD.

A total of 218 Thoroughbred weanlings on six central Kentucky farms were studied during December 1999 and January 2000. A glycemic response test was conducted by feeding a meal that consisted of the weanling's normal concentrate at a level of intake equal to 1.4 g nonstructural carbohydrate/kg body weight. A single blood sample was taken 120 min post feeding for the determination of plasma glucose and insulin concentrations. The glycemic index of each feed was also determined. Overall incidence of OCD on these farms was recorded until the horses were sold as yearlings.

Plasma glucose and insulin were significantly higher in weanlings with OCD than in unaffected foals. Foals that were heavier than average tended to be affected more often than foals that were lighter than average. There were strong positive correlations between mean glucose and insulin response on each farm and the incidence of OCD, and also between the glycemic index of each farm's feed and the farm's weanling glucose response. Based on the results of this study, it would be prudent to feed foals concentrates that produce low glycemic responses.

This research was published in *Proceedings of the American Association of Equine Practitioners Annual Convention*, 2001. ✦

Reproductive Efficiency of Thoroughbred Mares on Different Forage Regimens with Supplementation of Retinyl Palmitate and Beta-Carotene

K.M. Griewe-Crandell, D.S. Kronfeld, W.B. Ley, J.M. Bowen, and D. Sklan

Reproductive efficiency in the mare is lowest among domestic livestock and may be partly dependent on vitamin A status. To test this, 45 Thoroughbred mares were depleted of vitamin A for 8 months and then repleted for 20 months. During the repletion phase, mares were given retinyl palmitate, β-carotene, or a placebo. Foals were weighed at birth and monthly thereafter. Reproductive rates were calculated for the years in which depletion and repletion were developing. Retained placenta and contracted tendons were observed clinically.

During the repletion phase, supplementation of retinyl palmitate resulted in higher pregnancy rate, foaling rate and pregnancy loss than β-carotene. Lack of vitamin A supplementation during pregnancy may increase the risks of retained placenta and congenital contracted tendons. Mares appear to benefit from supplementation with vitamin A but not water dispersible β-carotene.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001. ✦

Influence of High Carbohydrate and High Fiber Diet on Mineral Metabolism in Horses

L. Krusic, J.D. Pagan, P. Schramel, M. Dermelj, and V. Stibilj

Twenty horses in five groups were used in this study. Horses were fed high carbohydrate or high fiber diets with or without added yeast. These diets were analyzed for mineral content. After a period of adaptation to the diets, a five-day collection was conducted for urine and feces. Urine, blood, and fecal samples were analyzed for mineral content. Intake and excretion levels of minerals were analyzed and correlated to dietary treatments.

Apparent digestibility of phosphorus tended to be higher in the high carbohydrate and high fiber unsupplemented groups. Apparent digestibility and retention of copper and selenium were significantly higher when feeding either of the yeast-supplemented diets. Apparent digestibilities of several other minerals were not significantly different between supplemented and unsupplemented diets. Plasma levels and renal excretion of some minerals was influenced by diet, though no difference was found for many minerals related to yeast supplementation.

This research was published in *Proceedings of the 17th Equine Nutrition and Physiology Society Symposium*, 2001.✦

Influence of Corrected Teeth on Daily Food Consumption and Glucose Availability in Horses

L. Krusic, J. Easley, and J.D. Pagan

Four groups of resting adult horses and a group of young horses in training participated in a 10-week feeding and exercise experiment. The diets of the resting group of horses (high fiber or high carbohydrate) were supplemented (or not) with a commercially available dietary yeast culture. The high fiber diet of the young exercising horses was yeast supplemented.

After an adaptation period of four weeks, all horses had their dental abnormalities corrected using a uniform method. Feed consumption did not meet the required 1% of body weight for hay and concentrates in resting groups or 1.5% in exercising group during the acclimation period. After dental correction, all horses consistently consumed their required amounts of feed and the rate of daily weight gain increased, beginning to level off during the end of the experiment.

Blood samples were taken prior to and four weeks after dental correction during the collection period. Blood glucose levels were compared between all horses prior to dental treatment during the acclimation period and six weeks later at the end of the experiment. The mean blood glucose concentrations increased significantly in two resting groups of horses, whereas in the unsupplemented high fiber diet group, there was only a slight significant increase in blood glucose. In the exercising group, mean blood glucose concentrations decreased slightly during the experiment.

This study was conducted in 2001. ✦

Effects of Fat Adaptation on Glucose Kinetics and Substrate Oxidation During Low-Intensity Exercise

J.D. Pagan, R.J. Geor, P.A. Harris, K. Hoekstra, S. Gardner, C. Hudson, and A. Prince

This study was designed to determine the effects of fat adaptation on carbohydrate and fat oxidation in conditioned horses during low-intensity exercise.

Five mature Arabians were studied. The study was conducted as a crossover design with two dietary periods, each of 10 weeks' duration: a) a control (CON) diet, and b) a fat-supplemented (FAT) diet. The total amount of digestible energy (DE) supplied by the fat in the CON and FAT diets was 7% and 29%, respectively. During each period, the horses completed exercise tests at the beginning of the period (Week 0) and after 5 and 10 weeks on the diet. Tests consisted of 90 min of exercise at a speed calculated to elicit 35% VO2max on a treadmill inclined to 3 degrees. Oxygen consumption (VO2), carbon dioxide production (VCO2), and respiratory exchange ratio (RER) were measured at 15-min intervals. For determination of glucose kinetics, a stable isotope ([6-6-d2] glucose) technique was used.

Compared to the CON diet, FAT diet consumption for 5-10 weeks was associated with an altered metabolic response to low-intensity exercise, as evidenced by a more than 30% reduction in the production and utilization of glucose; a decrease in RER; a decrease in the estimated rate of whole-body carbohydrate utilization; and an increase in the whole-body rate of lipid oxidation during exercise.

This research was published in *Equine Veterinary Journal*, 2002. ✦

Kentucky Foal and Yearling Growth Rates Not Affected by Mare Reproductive Loss Syndrome

J.D. Pagan

In the years directly after mare reproductive loss syndrome (MRLS) greatly reduced foal crops in central Kentucky, there was a concern that some aspect of the syndrome would affect growth rates of surviving foals and yearlings. This study compared the growth rates of Thoroughbred foals born in Kentucky in 2000 and 2001 against a large reference database compiled in the mid-1990s.

Body weights and wither heights were recorded for 435 foals raised on nine farms in central Kentucky. Average weights and wither heights were compared to baselines and found to be as good as or slightly higher than those found in previous years. It can be concluded that MRLS did not have a negative effect on growth of foals and yearlings.

This research was published by *Kentucky Equine Research* in 2002. ✦

Plasma and Urine Electrolyte and Mineral Concentrations in Thoroughbred Horses with Recurrent Exertional Rhabdomyolysis After Consumption of Diets Varying in Cation-Anion Balance

E.C. Mckenzie, S.J. Valberg, S.M. Godden, J.D. Pagan, G.P. Carlson, J.M. MacLeay, and F.D. DeLaCorte

This study was designed to determine whether plasma, urine, and fecal electrolyte and mineral concentrations differ between clinically normal horses and Thoroughbreds with recurrent exertional rhabdomyolysis (RER) after consumption of diets varying in cation-anion balance.

The study used 5 Thoroughbred mares with RER and 6 clinically normal mixed-breed mares. Each of 3 isocaloric diets designated as low, medium, and high on the basis of dietary cation-anion balance (DCAB) values of 85, 190, and 380, respectively, were fed to horses for 14 days. During the last 72 hours, 3 horses with RER and 3 control horses had daily urine and fecal samples obtained by total 24-hour collection. Remaining horses had urine samples collected daily by single catheterization.

For each diet, no differences existed between horses with RER and control horses in plasma pH, electrolyte concentrations, and creatine kinase activity or in urine pH and renal fractional excretion (FE) values. Plasma pH, strong ion difference, bicarbonate and total carbon dioxide concentrations, and base excess decreased and plasma chloride and ionized calcium concentrations increased with decreasing DCAB. Urine pH decreased with decreasing DCAB. The FE values of chloride and phosphorus were greatest for horses fed the low diet. The FE values for all electrolytes except magnesium did not differ between urine samples obtained by single catheterization and total 24-hour collection. Daily balance of calcium, phosphorus, sodium, chloride, and potassium did not differ significantly among horses fed the various diets. In clinically normal horses and in horses with RER, the DCAB strongly affects plasma and urine pH and the FE of sodium, potassium, chloride, and phosphorus.

This research was published in *American Journal of Veterinary Research*, 2002. ✦

Effect of Dietary Starch, Fat, and Bicarbonate Content on Exercise Responses and Serum Creatine Kinase Activity in Equine Recurrent Exertional Rhabdomyolysis

E.C. McKenzie, S.J. Valberg, S.M. Godden, J.D. Pagan, J.M. MacLeay, R.J. Geor, and G.P. Carlson

To determine the effect of dietary starch, bicarbonate, and fat content on metabolic responses and serum creatine kinase (CK) activity in exercising Thoroughbreds with recurrent exertional rhabdomyolysis (RER), 5 RER horses were fed 3 isocaloric diets (28.8 Mcal/d [120.5 MJ/d]) for 3 weeks in a crossover design and exercised for 30 minutes on a treadmill 5 days/wk. On the last day of each diet, an incremental standardized exercise test (SET) was performed. The starch diet contained 40% digestible energy (DE) as starch and 5% as fat: the bicarbonate-starch diet was identical but was supplemented with sodium bicarbonate (4.2% of the pellet): and the fat diet provided 7% DE as starch and 20% as fat.

Serum CK activity before the SET was similar among the diets. Serum CK activity after submaximal exercise differed dramatically among the diets and was greatest on the bicarbonate-starch diet (6.51 +/- 1.5) and lowest on the fat diet (5.71 +/- 0.6). Appreciable differences were observed in the severity of RER among individual horses. Postexercise plasma pH, bicarbonate concentration, and lactate concentration did not differ among the diets. Resting heart rates before the SET were markedly lower on the fat diet than on the starch diet. Muscle lactate and glycogen concentrations before and after the SET did not differ markedly among the diets.

This study showed that a high-fat, low-starch diet results in dramatically lower post-exercise CK activity in severely affected RER horses than does a low-fat, high-starch diet without measurably altering muscle lactate and glycogen concentrations. Dietary bicarbonate supplementation at the concentration administered in this study did not prevent increased serum CK activity on a high-starch diet.

This research was published in *Journal of Veterinary Internal Medicine*, 2003. ✦

Characteristics of Growth of Morgan Horses

L.A. Lawrence, H.R. Hearne, S.P. Davis, J.D. Pagan, A. Fitzgerald, and E.A. Greene

Growth data from 32 years of Morgan horse records were analyzed in this study.

Weights and wither heights were measured on foals every 45 days from day 145 to day 540. Weight and wither height data for males and females were pooled. The relationships among weight, height, and day of age were determined. Prediction equations were developed for weight, height, and age.

The horses in this study grew more slowly than NRC (1989) recommendations (50%) until day 462. From day 462 to day 540, the horses had average daily gains ranging from twice to three times the rates recommended by the NRC. Height at 18 months seemed to be unaffected by the average daily gain that diverged from the NRC from weaning until 18 months of age.

This report was published in the *Proceedings of the 18th Equine Nutrition and Physiology Society Symposium*, 2003. ✦

Potassium-Free Electrolytes and Calcium Supplementation in an Endurance Race

T.M. Hess, K. Greiwe-Crandell, D.S. Kronfeld, J.E. Waldron, C.A. Williams, M.A. Lopes, R.M. Hoffman, K. Gay, D. Ward, and P.A. Harris

Water intake and supplementation with electrolytes is crucial in the prevention of dehydration and metabolic problems during endurance races. This study was designed to test whether an experimental concentrate rich in fat and fiber (EF) and a potassium-free electrolyte mixture (EM-K) would have metabolic advantages in an 80 km race over an experimental concentrate rich in starch and sugar (ES) or commercial concentrates (CS) fed with or without commercial potassium-rich mixtures (EM+K).

In the study, forty horses were weighed and blood samples were taken before, during, and after the ride. The EM-K horses were less dehydrated than EM+K horses indicated by the lower plasma albumin and total protein at 80 km and after the race. Higher plasma sodium at 80 km and after the race would attract more water into the circulating blood, and these shifts help to maintain plasma volume and blood pressure. The lower plasma potassium found in the EM-K group and the higher calcium in the ES and EF supplemented horses may help prevent increases in neuromuscular excitability and related clinical signs. Less dehydration in the EM-K group would help maintain temperature regulation and delay fatigue.

This research was published in *Proceedings of the 18th Equine Nutrition and Physiology Society Symposium*, 2003. ✦

Comparison of Volumetric Urine Collection Versus Single-Sample Urine Collection in Horses Consuming Diets Varying in Cation-Anion Balance

E.C. McKenzie, S.J. Valberg, S.M. Godden, J.D. Pagan, G.P. Carlson, J.M. MacLeay, F.D. De La Corte

This study determined daily variation in urinary clearance and fractional excretion (FE) of electrolytes and minerals within and between horses, and compared volumetric and single-sample urine collection for determining FE values of diets with a range of dietary cation-anion balance (DCAB).

Five Thoroughbred and 6 mixed-breed mares were used. Three isocaloric diets with low, medium, and high DCAB values (85, 190, and 380 mEq/kg of dry matter, respectively) were each fed for 14 days. Daily blood samples, single urine samples collected by using a urinary catheter (5 mares), and volumetric urine collections (6 mares) were obtained during the last 72 hours of each diet. Urine and plasma pH values, plasma concentrations, and FE values of sodium, chloride, potassium, magnesium, phosphorus, and calcium were altered by varying the DCAB. Noticeable variation in clearance and FE values was detected within horses from day to day on the same diet as well as between horses. Fractional excretion values were not significantly different between single-sample and volumetric methods, except for magnesium in the high DCAB diet. Volumetric and single-sample collections revealed similar patterns of change in urinary FE values with varying DCAB, except for calcium and magnesium.

This study showed that substantial variations in clearance and FE of electrolytes and minerals were evident within horses between 24-hour periods as well as between horses fed a specific diet. Three daily urine samples provided similar information regarding dietary-induced changes in clearance and FE values (excluding calcium and magnesium) as that obtained by volumetric urine collection.

This research was published in *American Journal of Veterinary Research*, 2003. ✦

Comparison of Vitamin E Blood Levels in Horses Fed Three Different Sources of Vitamin E

J.D. Pagan

The study determined vitamin E blood levels in horses fed three different sources of vitamin E.

Nine mature unexercised Thoroughbred geldings were divided into three groups and supplemented with synthetic (SYN), natural-source (NAT), or micellized water-soluble vitamin E (WS). Horses were maintained on a diet of unfortified sweet feed and grass hay and were muzzled during turnout to prevent grazing. Baseline blood samples were taken following 14 days of no supplementation. Horses were then supplemented with 500 IU of vitamin E from the assigned treatment source for 14 days. The dose rate of vitamin E was doubled at 14-day increments up to 8,000 IU. Plasma tocopherol levels were measured at the end of each 14-day period. Plasma tocopherol levels were significantly elevated from the baseline in the WS and NSAT groups at 1,000-8,000 IU, respectively.

No significant difference was recorded in the SYN group as dose rate was increased. Significant differences among groups were recorded at various dosage rates, with WS always at highest and SYN always at lowest levels.

This research was conducted in 2004. ✦

The Effect of Varying Dietary Starch and Fat Content on Serum Creatine Kinase Activity and Substrate Availability in Equine Polysaccharide Storage Myopathy

W.P. Riberto, S.J. Valberg, J.D. Pagan, and B. Essen-Gustavsson

Polysaccharide storage myopathy (PSSM) is a genetic disease that can cause muscle cramping and stiffness. The purpose of this exercise trial was to determine the effects of four diets varying in starch and fat content on blood glucose and insulin concentrations as well as on indicators of exercise-induced muscle strain and damage in horses with PSSM.

Horses had greater blood glucose and insulin responses when they were fed a traditional high-starch diet (sweet feed composed of 45% corn, 45% oats, and 10% molasses). As the proportion of starch was lowered and proportions of fat and fiber were raised in the diet, blood glucose and insulin responses after a meal decreased. The most even plane of energy use was found among horses eating RE-LEVE®, a low-starch, high-fat commercial concentrate made of soy hulls, rice bran, soybean, corn oil, wheat, and pellet binder. While the amount of post-exercise muscle stiffness varied among horses on all diets, horses on the high-starch diet exhibited the most muscle stiffness and those on the RE-LEVE® diet exhibited the least muscle stiffness.

Results of this study indicate that horses with polysaccharide storage myopathy benefit from diets such as RE-LEVE® that are lower in starch and higher in fat and fiber than traditional sweet feeds. Low-starch, high-fat feeds tend to minimize large fluctuations in blood glucose, and horses eating these rations will experience fewer and less severe episodes of muscle damage and stiffness.

Although the cause of muscle stiffness in horses with PSSM is not completely understood, simple dietary modification can allow these horses to train and perform with little or no discomfort.

This research was published in *Journal of Veterinary Internal Medicine,* 2004. ✦

Size Matters at the Sales

J.D. Pagan, A. Koch, and S. Caddel

Buyers look at various factors—pedigree, conformation, and racing performance of siblings—when considering the purchase of a Thoroughbred yearling. In order to find out the influence of size on sale price, this study considered size as a factor in sale price for horses sold at the 2003 Keeneland September Yearling Sale.

Body weight, wither height, and body condition score measurements were taken in late August and early September for 294 yearlings to be sold. Session and sale price were recorded and correlated to price. The yearlings were from 16 farms and were sold by 20 consignors.

Sale prices (or last bid for horses not sold) were used to divide the horses into two groups based on whether the horse sold above or below the session's median price. Sold and unsold yearlings were also compared. Body weight was significantly greater in both colts and fillies that received bids above their session median. Horses that did not sell tended to be lighter than horses listed as sold, and many unsold horses also had lower body condition scores.

This research was conducted in 2004 and published in *Advances in Equine Nutrition IV*. ✦

The Efficiency of Utilization of Digestible Energy During Submaximal Exercise

J.D. Pagan, G. Cowley, D. Nash, A. Fitzgerald, L. White, and M. Mohr

Using the factorial and global methods of calculating energy requirements, a study was carried out to determine the DE requirements for a measured amount of exercise; to determine the efficiency with which DE was utilized for work; and to develop a new equation to predict DE requirements for exercise.

Results suggest the efficiency of utilization of DE by horses during work is significantly lower than during rest, which is contradictory to the assumptions currently used to calculate energy requirements for work by the factorial method.

More research is needed to determine the factors that affect this efficiency.

This research was conducted in 2004 and published in *Advances in Equine Nutrition IV*. ✦

Form and Source of Tocopherol Affects Vitamin E Status in Thoroughbred Horses

J.D. Pagan, E. Kane, and D. Nash

The purpose of this research was to assess the effects of different forms of dietary vitamin E supplementation on vitamin E status. Nine horses were supplemented for 56 days with 5,000 IU of vitamin E in one of three forms: synthetic vitamin E (dl-alpha-tocopherol acetate), natural-source vitamin E (d-alpha tocopherol acetate), and natural vitamin E (d-alpha-tocopherol) in a micellized water-soluble form. Blood samples were taken prior to supplementation and at weekly intervals during the supplementation period for measurement of plasma tocopherol levels.

After supplementation, plasma tocopherol levels were significantly higher in horses receiving natural-source vitamin E than in those receiving synthetic vitamin E. The highest plasma tocopherol levels were found in horses receiving natural vitamin E in a micellized water-soluble form.

Results of this research indicate that the source of vitamin E significantly affects vitamin E status in exercised and unexercised horses. Synthetic vitamin E (dl-alpha-tocopherol acetate) was less effective at elevating plasma tocopherol levels than natural solurce vitamin E, and a micellized form of vitamin E was superior at elevating plasma tocopherol during short-term administration.

This research was published in *Pferdheikunde,* 2005.✦

Size of Thoroughbred Yearlings Presented for Auction at Keeneland Sales Affects Selling Price

J.D. Pagan, A. Koch. S. Caddel, and D. Nash

Buyers look at various factors—pedigree, conformation, and racing performance of siblings—when considering the purchase of a Thoroughbred yearling. In order to find out the influence of size on sale price, this study considered size as a factor in sale price for horses sold at the 2003 and 2004 Keeneland September Yearling Sales.

Body weight, wither height, and body condition score measurements were taken in late August and early September for 630 yearlings to be sold. Session and sale price were recorded and correlated to price. The yearlings were from 23 farms.

Sale prices (or last bid for horses not sold) were used to divide the horses into two groups based on whether the horse sold above or below the session's median price. Further division was made by gender. Sold and unsold yearlings were also compared. Body weight was significantly greater in both colts and fillies that received bids above their session median. Wither height was not significant for colts, but for fillies, those with greater wither height sold better. Horses that did not sell tended to be shorter than horses listed as sold. In summary, yearlings that commanded bids higher than the median price of the session in which they were sold tended to be heavier and taller, but not fatter, than yearlings receiving bids below the session's median price.

This research was published in *Proceedings of the 19th Equine Nutrition and Physiology Society Symposium*, 2005. ✦

Body Weight, Wither Height, and Growth Rates in Thoroughbreds Raised in America, England, Australia, New Zealand, and India

C.G. Brown-Douglas, J.D. Pagan, P.J. Huntington, and G. Jenkinson

The purpose of this study was to obtain growth data from populations of Thoroughbreds born and raised in various countries and compare body weight, daily weight gain, and wither height growth curves. Information was collected for the years 1996 to 2006.

A linear regression model with foal age as the explanatory variable and country as the response variable was used to predict weight, height, and average daily gain and provide a confidence interval for the mean response at a specified age within each month of age category.

Significant differences in body weight, wither height, and average daily gain were observed between the populations of Thoroughbreds. In general, Australian and New Zealand Thoroughbreds tended to be larger than American Thoroughbreds, which in turn were larger than those reared in England. Thoroughbreds raised in India tended to be smaller than those born in other countries.

This research was conducted in 2006 and published in A*dvances in Equine Nutrition IV*. ✦

Body Weight and Condition of Kentucky Thoroughbred Mares and Their Foals as Influenced by Month of Foaling and Season

C.G. Brown-Douglas, J.D. Pagan, and S. Caddel

Several studies have assessed the effect of birth month and season on foal body weight; however, none of these studies evaluated the effect of season on changes in body weight or body condition in the lactating mare.

Thoroughbred mares (n=3909) and their foals were weighed monthly to assess the influence of month of foaling, season, and gender on body weight, body condition score, and daily weight gain.

Colts were heavier and taller than fillies throughout the study. After one month of age, fillies were fatter than colts. Foals born in January and February were smaller at birth and grew more slowly early in life, but by 150 days of age this lag disappeared. Mare weight changes and body condition scores were related to season and management factors, as winter-foaling mares lost weight and had lower body condition post foaling than spring-foaling mares.

This research was conducted in 2006 and published in *Advances in Equine Nutrition IV*.✦

The Influence of Body Weight, Wither Height and Body Condition Score on Sale Price of Thoroughbred Yearlings at Public Auction

J.D. Pagan, D.M. Nash, A. Koch, S. Caddel, and C.G. Brown-Douglas

Buyers look at various factors—pedigree, conformation, and racing performance of siblings—when considering the purchase of a Thoroughbred yearling. This study was conducted to find out the influence of body size and condition score on sale price.

Body weight, wither height, and body condition score measurements were taken in late August and early September for 630 yearlings to be sold at the 2003 and 2004 Keeneland yearling sales. Session and sale price were recorded and correlated to price.

Sale prices were used to divide the horses into two groups based on whether the horse sold above or below the session's median price. Further division was made by age and gender. Sold and unsold yearlings were also compared. Body weight was significantly greater in both colts and fillies that received bids above their session median. When age and gender were considered, weight was significantly greater but wither height was not significantly greater for horses that sold better. Horses that did not sell were taller but not heavier than those that sold when adjusted for age and gender. These data suggest that the ideal condition score for a sales yearling is 6.0 based on a scale of 1 through 9. Yearlings presented for sale with lower condition scores may be less likely to meet sellers' expectations.

This research was published in *Proceedings of the 20th Equine Science Society Symposium*, 2007. ✦

Thoroughbred Growth and Future Racing Performance

C.G. Brown-Douglas, J.D. Pagan, and A.J. Stromberg

Growth measurements were made on 3,734 Thoroughbreds raised in the U.S. between 1996 and 2002. Racing performance data were collected for these horses and their growth records were retrospectively examined to determine if various growth characteristics could be associated with success as a racehorse. To account for genetic variation, the sire index was collected for each horse to indicate the average racing class of foals sired by a particular stallion.

Of the 3,734 foals, 79% started in a race and 71% of those won at least one race. More colts raced than fillies, and more colts won at least one race. There was no difference in body weight percentile or height percentile as foals, sucklings, and weanlings between horses that were raced versus unraced. Yearlings that raced had significantly lower average weight percentiles than those that did not. Horses that raced as two-year-olds were significantly shorter and lighter than those that did not start as two-year-olds in all age groups. There was no significant difference in body weight percentile or wither height percentile between winners and non-winners in all age groups. Sire index of graded stakes winners was significantly greater in both foals and yearlings than those that did not win a graded stakes race. Horses in the lowest weight quartile at all age groups had significantly more starts than those in the upper weight quartile. In foals, height percentile was the only variable included in the model that significantly predicted the probability of being a stakes winner, with taller foals having a better chance.

Tall but not heavy young growing horses are more likely to become successful athletes. It is recommended that young growing horses be weighed and measured to ensure the skeleton maintains a steady rate of growth while preventing the animal from becoming too heavy.

This research was conducted in 2006 and published in *Advances in Equine Nutrition IV*. ✦

Body Weight and Growth Rates in Australian Thoroughbreds Compared with Thoroughbreds in Kentucky, England, New Zealand and India

C.G. Brown-Douglas, J.D. Pagan,
P.J. Huntington, and G. Jenkinson

Growth characteristics including body weight, height, and average daily gain were measured between birth and 18 months of age in Thoroughbred horses born and raised on commercial stud farms in Australia, New Zealand, Kentucky, England, and India during the years 1996 to 2005. Australian and Kentucky foals were heavier at 7 days of age than those born in the other countries, with Indian foals being lightest.

Body weight did not differ between Australian, New Zealand, and Kentucky Thoroughbreds between 1 and 18 months, but Australian and New Zealand yearlings tended to be heavier than the other populations between 12 and 18 months.

This research was published in P*roceedings of the Australasian Equine Science Symposium*, 2006. ✦

The Effects of a Time-Released Sodium Bicarbonate Buffer on Exercising Horses Receiving High Concentrate Diets

J.D. Pagan and T.J. Lawrence

Modern feeding practices for performance horses often include large grain meals, a diet that can lead to hindgut acidosis. Buffering the hindgut can be an effective prevention of subclinical acidosis, but delivering the buffer to the affected area has proven difficult. This study was designed to evaluate the effect of EquiShure®, a hindgut buffer developed by Kentucky Equine Research, on blood and hindgut parameters in horses receiving high grain diets.

Seven fit Thoroughbred geldings were used in a switchback design with periods of four weeks. Horses received unfortified sweet feed, timothy grass hay, and 50g of loose salt, either with or without 168g/day of EquiShure. Blood and fecal samples were analyzed for pH. Volatile fatty acids (VFA) were analyzed as an indicator of microbial metabolism in the hindgut.

Horses receiving EquiShure® supplementation exhibited VFA patterns that demonstrated the attenuating effects the time-released buffer appears to have on the hindgut environment, thereby minimizing dramatic shifts in pH.

This research was conducted in 2007. ✦

Feeding Protected Sodium Bicarbonate Attenuates Hindgut Acidosis in Horses Fed a High-Grain Ration

J.D. Pagan, T.J. Lawrence, and L.A. Lawrence

Hindgut acidosis is a common problem in horses consuming large quantities of either grain or fructan-rich forages. This dietary regimen may lead to shifts in hindgut acidity when a portion of the grain starch is not digested in the small intestine. Passing into the cecum and colon, the starch is rapidly fermented, changing the environment of the hindgut. Horses suffering from this condition may develop anorexia, colic, or laminitis, and may develop behaviors like wood chewing, weaving, and stall walking. This study was designed to test the efficacy and safety of feeding a protected sodium bicarbonate product to treat hindgut acidosis in horses fed a high-grain ration.

Six horses in good physical condition were split into two treatment groups. All horses were fed hay and a high-grain ration for a period of four weeks. In addition, horses in one group were given protected sodium bicarbonate product, while horses in the other group served as a control. The horses were exercised daily on a high-speed treadmill. Analysis of fecal samples showed that control horses developed hindgut acidosis by six hours after feeding, while horses given the protected sodium bicarbonate did not show decreasing fecal pH during the same period.

Horses in training frequently require large grain meals to provide energy for performance. Results of this study indicate that feeding a protected sodium bicarbonate produce (EquiShure®, Versailles, KY) to horses on high-grain diets can attenuate the pH shifts that lead to hindgut acidosis. Coating the sodium bicarbonate product allows it to reach the hindgut in an active state instead of being neutralized in the stomach and small intestine. Horses receiving a protected sodium bicarbonate product are protected from the discomfort, health threats, and undesirable habits associated with hindgut acidosis.

This research was published in *Proceedings of the American Association of Equine Practitioners Conference*, 2007. ✦

The Relationship Between Size at Yearling Sale, Sale Price, and Future Racing Performance in Kentucky Thoroughbreds

C.G. Brown-Douglas, J.D. Pagan, A. Koch, and S. Caddel

Previous research has shown a relationship between yearling size and future racing performance. Yearlings that weighed in the lowers 25% of the population had lower earnings and fewer stakes winners than the rest of the population. Yearlings below the 50th weight and height percentiles were more likely to start as two-year-olds and had more career starts than larger horses. This study examined body size and selling price of yearlings and compare these with results from a study of yearling size and future racing performance.

Body weight and wither height were recorded for 1040 yearlings sold in 2003-2006. They were divided into groups according to whether they sold above or below the median price for their sales group. Larger and taller yearlings sold better than smaller horses.

Correlations of sale price to race earnings showed that yearlings in the second weight quartile had the most stakes winners and greatest career earnings. Heavier yearlings sold for greater amounts but had fewer stakes wins and overall earnings. The tallest yearlings sold for less but had greater career earnings and number of stakes wins.

This research was published in P*roceedings of the 20th Equine Science Society Symposium*, 2007. ✦

The Relationship Between Bucked Shins, Blood Parameters and Cannon Bone Measurements in Thoroughbreds Being Prepared for Two Year Olds in Training Sales

J.D. Pagan, L.A. Lawrence, and D. Nash

Bucked shins is a term used to describe a condition of the third metacarpal bone which is related to bone fatigue and/or stress fractures. This is a common problem in racehorses in the first year of training. Thirty Thoroughbreds were studied as they were prepared for two-year-olds in training sales that took place in late winter or early spring. Measurements began when the horses were already in moderate to intense training and lasted 56 days. Bone mineral content was calculated and bone morphological measurements were measured from radiographs. Plasma concentrations of calcium, phosphorus, and osteocalcin were also measured.

There was a trend toward greater bone mineral content and radiographic bone aluminum equivalency in the unaffected horses, and these horses also had significantly greater lateral cortical width, bone width, and total radiographic bone aluminum equivalency. Plasma calcium, phosphorus, and osteocalcin dropped in affected (5) and unaffected (25) horses throughout the study. Calcium and phosphorus levels were similar between the groups but osteocalcin was significantly higher in affected horses. The explanation for these differences is not apparent, since all the horses in the study were managed similarly.

This research was published in *Proceedings of the Australasian Equine Science Symposium*, 2007. ✦

Skeletal Adaptations with the Onset of Training in Thoroughbreds

J.D. Pagan, L.A. Lawrence, and D. Nash

Previous studies have found a decrease in the mineral content of the third metacarpal bone during the first months after a young horse leaves a breeding farm environment and enters race training. To explore the skeletal adaptations involved in early race training, bone density and morphometry were tracked in 15 Thoroughbred yearlings as they began training at a facility where turnout paddocks were available.

The horses were stalled for about 6 hours per day and training consisted of 15–20 minutes of slow jogging per day. After the first month they were moved to a facility without turnout where the horses were stalled for 23 hours each day. They were lightly exercised for two months and then began training more intensely.

During training, dorsopalmar radiographs of the third metacarpal bone were taken on a monthly basis and an aluminum step wedge was exposed simultaneously as a reference standard. Plasma concentrations of calcium, phosphorus, and osteocalcin were also measured monthly.

Bone mineral content dropped while the horses were confined to stalls with only light exercise. When training intensity increased, bone mineral content also increased. Plasma concentrations of calcium, phosphorus, and osteocalcin followed similar patterns.

This research was published in *Proceedings of the 20th Equine Science Society Symposium*, 2007. ✦

Relationship Between Body Condition and Metabolic Parameters in Sport Horses, Pony Hunters and Polo Ponies

J.D. Pagan, O.A. Martin, N.L. Crowley, and K.L. Hooks

Obesity has been associated with insulin resistance in horses and ponies. Performance horses such as racehorses, endurance horses, and polo ponies are usually kept in relatively lean body condition, but many sport horses such as dressage horses, show hunters, and show jumpers carry greater body condition. This study was conducted to quantify whether body condition was related to resting insulin, glucose, and triglycerides.

Using 181 horses and ponies in training for the winter show season in Florida, researchers recorded body weight, wither height, neck circumference, body condition score, and neck crest adiposity. Blood samples were taken before each horse's morning meal and analyzed for plasma glucose, insulin, and triglycerides.

Results showed that dressage horses, show hunters, and show jumpers carried significantly more body condition than polo ponies, but had similar resting insulin, glucose, and triglyceride values. Pony hunters were significantly fatter than dressage horses, show hunters, show jumpers, and polo ponies, and had significantly higher resting insulin and significantly lower resting plasma glucose values. However, it appeared that overweight sport horses and ponies were less likely to be hyperinsulinemic than sedentary horses and ponies, perhaps because of their training regimen.

This research was published in *Journal of Equine Veterinary Science,* 2008. ✦

A Comparison of Actual and Recommended Digestible Energy Intakes Derived from Differing Feeding Standards for Miniature Horses

A.L. Scherer-Hoock, E.A. Greene, M. Lennox, and C. Brown-Douglas

The purpose of this study was to determine if feeding recommendations for full-sized horses could be linearly scaled down to accurately reflect the digestible energy intake required by miniature horses maintaining a healthy weight and body condition score.

Twelve miniature horses considered to be of ideal weight and body condition were selected as a reference group, and their daily feed intake, body weight, and body condition were recorded. Dietary energy in each horse's diet was calculated and these numbers were compared to suggested feeding guidelines from several sources. Actual requirements for dietary energy in this group were lower than suggested guidelines from other sources.

With obesity common among miniature horses, more research is needed, which could potentially lead to the computation of new miniature horse-specfic feeding recommendations that would provide a better foundation for formulating rations for these horse horses, thereby reducing the instance of overfeeding and its negative effects.

This report *Journal of Equine Veterinary Science*, 2009. ✦

Glycemic/Insulinemic Response to Feeding Hay with Different Nonstructural Carbohydrate Content in Control and Polysaccharide Storage Myopathy-Affected Horses

L. Borgia, S. Valberg, K. Watts, and J.D. Pagan

The objectives of this study were to determine if there is a difference in the glycemic/insulinemic response to hay with high or low nonstructural carbohydrate (NSC) content in horses of Quarter Horse or related breeding, and to determine if there is a difference in the postprandial response to hay with high or low NSC in control horses compared to PSSM horses, all of Quarter Horse-related breeds.

A crossover design was used with horses randomly assigned to high-NSC (HC) or low-NSC (LC) hay for 5 days, then fed a medium-NSC (MC) hay during the 7-day washout, after which diets were reversed. Horses were fed 1.5% body weight (BW) hay daily. On the fifth day of the diet (seventh day of washout), horses were fed 0.5% BW in hay, and intake rate was recorded. Blood was drawn pre-feeding and every 30 minutes for 5 hours for glucose and insulin.

HC hay had the fastest intake rate. When fed HC vs. LC hay, control horses had a similar glycemic but higher insulinemic response. On HC hay, PSM horses had a higher glycemic and lower insulinemic response than controls.

Results from this study suggest that glucose alone might not accurately reflect insulin responses to these feeds and that a glycemic index might not capture the complete relationship between NSC contents and metabolic impact of feeds. Postprandial insulin response, rather than glucose response, might better assess metabolic impact of hay. Hay with more than 17% NSC may not be appropriate for horses diagnosed with PSSM.

This research was published in *Journal of Equine Veterinary Science*, 2009. ✦

Effect of Triheptanoin on Muscle Metabolism During Submaximal Exercise in Horses

M.E. McCue, S.J. Valberg, J.D. Pagan, B. Essen-Gustavsson, and C.R. Roe

The goal of this study was to compare effects of corn oil or a 7-carbon fat (triheptanoin) on acylcarnitine, lipid, and carbohydrate metabolism in plasma or muscle of exercising horses.

Eight Thoroughbred geldings were given isocaloric diets containing 650 mL of oil (triheptanoin or corn oil)/d for 18 or 25 days in a crossover design with a 26-day washout period. On day 17 or 24 of each feeding period, the respective oil (217 mL) was nasogastrically administered, and 120 minutes later, horses performed a 90-minute submaximal exercise test (SET). Blood and muscle samples were obtained before oil administration and immediately before (blood only), during (blood only), immediately after, and 24 hours after SETs.

Compared with values before oil administration, triheptanoin administration increased plasma insulin and C7:0-, C5:0- and C3:0-acylcarnitine concentrations, whereas corn oil administration increased plasma NEFA concentrations. During SETs, plasma C7:0-, C5:0-, and C3:0-acylcarnitine concentrations were higher when triheptanoin, rather than corn oil, was administered to horses. Plasma glucose, NEFA, and C2:0-, C18:1-, and C18:2-acylcarnitine concentrations increased during SETs similarly for both oils. Respiratory quotient and muscle lactate, citrate, malate, glycogen, and ATP concentrations changed similarly from before to after SETs for both oils. Compared with muscle concentrations immediately after SETs, those for glucose-6-phosphate and citrate 24 hours after SETs were lower and for glycogen were similar to values before SETs. Fatigue was not associated with depletion of citric acid cycle intermediates for either oil. Triheptanoin induced a significantly higher insulin secretion and did not appear to enhance muscle glycogen repletion.

This research was published in *American Journal of Veterinary Research*, 2009. ✦

The Effect of Short-Term Adaptation to a High-Fat Diet on Insulin Sensitivity in Aged Thoroughbred Horses

L. Perry, J.D. Pagan, and L. Wood

Insulin resistance is an associated risk factor in laminitis, equine metabolic syndrome, and equine Cushing's disease, with aged horses at particular risk. This study was designed to examine whether short term adaptation to a high fat diet would affect insulin sensitivity in aged horses.

During the three-period study, three Thoroughbred geldings were given mixed grass/legume hay and unfortified sweet feed for the first and third periods. In the second period they were given the same amount of hay plus hay cubes and 600 ml of soybean oil. After administration of a dextrose solution by nasogastric tube, glucose tolerance tests were conducted. This was done on days 28 and 35 of each period.

Glucose was higher in the FAT group at 120 minutes compared to horses on the carbohydrate diet. Insulin in the FAT group was significantly lower at 5, 60, 90, and 120 minutes compared to horses on the carbohydrate diet. Horses on the high fat diet produced less insulin and took longer to clear glucose from their blood. The results of this study suggest that feeding high fat to aged horses reduced insulin sensitivity compared to a moderate high carbohydrate diet.

This KER research was conducted in 2010. ✦

Fish Oil and Corn Oil Supplementation Affect Red Blood Cell and Serum Eicosapentaenoic Acid (EPS) and Docosahexaenoic Acid (DHA) Concentrations in Thoroughbred Horses

J.D. Pagan, T.L. Lawrence, M. Lennox, and C.G. Brown-Douglas

Horses require both omega-3 and omega-6 fatty acids in their diets. Long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are intermediate in the formation of eicosanoids that have been shown to reduce inflammatory responses, support immune function, and enhance fertility. This study was conducted to compare the effect of supplementation with oil high in EPA and DHA (fish oil) or low in EPA and DHA (corn oil) on red blood cell (RBC) and serum EPA and DHA.

Twelve Thoroughbred geldings were supplemented for 127 days with 60 ml of either fish oil (EO-3) or corn oil. Blood samples were taken at days 0, 29, 57, 92, and 127 and analyzed for EPA and DHA. By day 29, horses receiving fish oil had an average increase in serum EPA and DHA of 3.7-fold and 17.9-fold, respectively. In horses receiving corn oil, serum EPA decreased 1.5-fold from baseline at day 57 and fourfold by day 92. By day 127, RBC DHA concentrations in the fish oil supplemented horses was over 1.9-fold greater than baseline, while there was no significant difference observed in RBC DHA from horses receiving corn oil. In the fish oil supplemented group, RBC EPA increased 11.5-fold by day 127. Corn oil supplemented horses had significantly lower than baseline RBC EPA at 57, 92, and 127 days.

Results of this study showed that 60 ml/d of fish oil supplementation (EO-3) significantly increases both serum and RBC EPA and DHA in horses. Corn oil supplementation resulted in a significant decrease in RBC EPA, which may affect RBC membrane fragility.

This research was published in *Proceedings of the Australasian Equine Science Symposium*, 2010. ✦

Form of Alpha-Tocopherol Affects Vitamin E Bioavailability in Thoroughbred Horses

J.D. Pagan, M. Lennox, L. Perry, L. Wood, L.J. Martin, C. Whitehouse, and J. Lange

Vitamin E can be obtained from natural or synthetic sources, but the chemical structure of each is different. The following studies were conducted to determine if synthetic and natural-source vitamin E have similar bioavailabilities when administered at equal IU doses and to determine if water dispersible forms of vitamin E are more bioavailable than lipid-soluble forms.

In study 1, single 5,000-IU oral doses of three different forms of vitamin E were evaluated in eight Thoroughbreds during three one-week periods. The forms tested were synthetic vitamin E (dl-alpha-tocopheryl acetate) (SYN); natural-source vitamin E acetate (d-alpha-tocopheryl acetate) (ACT); and natural-source alcohol (d-alpha-tocopherol) (ACL). Baseline blood serum samples were collected immediately before dosing and at 3, 6, 9, 12, and 24 hours post-dosing. In this study, ACT and ALC had a significantly greater AUC than SYN. There was no significant difference in AUC between ACT and ALC. Relative to SYN, the bioavailability of ACT and ALC equaled 197% and 252%, respectively.

In study 2, three Thoroughbreds were used in a replicated 3 x 3 Latin square design trial to assess the relative bioavailability of 5,000-IU oral doses of synthetic vitamin E (dl-alpha-tocopheryl acetate) (SYN); a micellized d-alpha-tocopherol; and a d-alpha-tocopherol (Nano-E®) that had been nanodispersed into liposomes. Baseline blood serum samples were collected immediately before dosing and at 3, 6, 9, 12, 24, 36, and 48 hours post-dosing. The micellized d-alpha-tocopherol and Nano-E had a significantly greater AUC than SYN. There was no significant difference in AUC between the micellized d-alpha-tocopherol and Nano-E. Relative to SYN, the bioavailability of the micellized d-alpha-tocopherol and Nano-E equaled 559% and 613%, respectively. Nano-E had significantly higher peak and maximal change from baseline values compared to SYN.

The results of these studies suggest that natural sources of vitamin E have a greater bioavailability than is accounted for in the current conversion factors of 1.36 and 1.49 used in the feed industry for natural acetate and alcohol, respectively. Nano-E, a natural-source, water-dispersible form of vitamin E, was five to six times more bioavailable than synthetic vitamin E acetate, and a 5000-IU dose more than doubled serum vitamin E levels within 12 hours.

This research was published in P*roceedings of the First Nordic Feed Science Conference*, 2010. ✦

Effect of Fitness on Glucose, Insulin and Cortisol Responses to Diets Varying in Starch and Fat Content in Thoroughbred Horses with Recurrent Exertional Rhabdomyolysis

C.J. Finno, E. McKenzie, S.J. Valberg, and J.D. Pagan

Recurrent exertional rhabdomyolysis (RER) occurs in fit, nervous Thoroughbreds fed high nonstructural carbohydrate (NSC) diets. Clinical signs are diminished by feeding low NSC, high fat diets; however, the mechanism is unclear. This study was performed to determine if the glucose, insulin, and cortisol responses to isocaloric diets varying in fat and NSC availability differ in fit vs. unfit Thoroughbreds with RER.

Four fit RER Thoroughbred mares were exercised and fed 3 isocaloric diets in a 5 day/diet block design. Two high-NSC concentrates, sweet feed (SF), and a processed pelleted feed (PL) and a low starch, high fat feed (FAT) were used. After 24 h of rest and a 12 h fast, horses ate half their daily concentrate. Blood sampled for glucose, insulin, and cortisol was obtained before, immediately after, and at 30-60 minute intervals for 420 minutes. After a 3-6 month detraining period, the block design was repeated.

Results for SF and PL were similar. Regardless of diet, cortisol was higher in fit vs. unfit horses. Fit horses on SF/PL had higher post-prandial insulin and insulin:glucose ratios than unfit horses. FAT resulted in lower post-prandial glucose and insulin vs. SF/PL. Higher insulin in fit vs. unfit horses was not seen on the FAT diet. Increased post-prandial glucose, insulin, and cortisol induced by high NSC, but not high fat, feeds are enhanced by fitness in RER horses. This combination may trigger rhabdomyolysis through increased excitability in RER Thoroughbreds.

This research was published in *Equine Veterinary Journal*, 2010. ✦

Effect of Dietary Fats with Odd or Even Numbers of Carbon Atoms on Metabolic Response and Muscle Damage with Exercise in Quarter Horse-Type Horses with Type 1 Polysaccharide Storage Myopathy

L.A. Borgia, S.J. Valberg, M.E. McCue, J.D. Pagan, and C.R. Roe

The objective of this study was to evaluate the effects of fats with odd and even numbers of carbon atoms on muscle metabolism in exercising horses with polysaccharide storage myopathy (PSSM).

The study used eight horses with PSSM (6 females and 2 males; mean +/- SD age, 6.3 +/- 3.9 years). Isocaloric diets (grain, triheptanoin, corn oil, and high-fat, low-starch [HFLS] feed) were fed for 3 weeks each. Horses performed daily treadmill exercise. Grain was fed to establish an exercise target, and HFLS feed was fed as a negative control diet. Daily plasma samples were obtained. For each diet, a 15-minute exercise test was performed, and gluteus medius muscle specimens and blood samples were obtained before and after exercise.

Feeding triheptanoin, compared with the corn oil diet, resulted in exercise intolerance; higher plasma creatine kinase (CK) activity and concentrations of C3:0- and C7:0-acylcarnitine and daily insulin; and lower concentrations of nonesterified fatty acids (NEFA) and C16:0-, C18:1-, and C18:2-acylcarnitine, without changes in concentrations of plasma glucose or resting muscle substrates and metabolites. Feeding grain induced higher CK activity and insulin concentrations and lower NEFA concentrations than did corn oil or HFLS feed. Feeding grain induced higher glucose concentrations than did triheptanoin and corn oil. In muscle, feeding grain resulted in lower glucose-6-phosphate, higher citrate, and higher postexercise lactate concentrations than did the other diets. It was concluded that triheptanoin had detrimental effects, reflecting decreased availability of NEFA, increased insulin stimulation of glycogen synthesis, and potential inhibition of lipid oxidation. Long-chain fats are the best dietetic for PSSM.

This research was published in the *American Journal of Veterinary Research*, 2010. ✦

Effect of Dextrose Supplementation on Electrolyte and Water Absorption in Resting Thoroughbreds

J.D. Pagan, B.M. Waldridge, J. Lange, C.G. Brown-Douglas, and P.J. Huntington

Commercial electrolyte products often contain dextrose, which is purported to improve electrolyte uptake in horses. Studies conducted at Kentucky Equine Research suggest that adding dextrose or starch to electrolyte mixes does not increase rate of absorption or retention of electrolytes. Dextrose may still have some value in improving palatability of electrolyte mixes, but the higher the dextrose content, the lower the electrolyte content of a product. This means high dextrose products supply lower amounts of electrolytes per kg, and may be less effective as a result.

This research was published in *Proceedings of the Australasian Equine Science Symposium*, 2012. ✦

The Effect of DuraPlex® Supplementation on Bone Density in Thoroughbred Horses Subjected to Either an Increasingly Strenuous Exercise Program or Complete Stall Confinement

J.D. Pagan

The objective of this study was to determine if DuraPlex®, a proprietary blend of nutrients designed to improve bone density in horses, would affect bone density in Thoroughbreds subjected to paddock turnout, treadmill exercise, or complete stall confinement.

Results of this study suggest that forced exercise on a treadmill and mechanical walker is adequate to maintain bone density in Thoroughbred horses. Total stall confinement may lead to bone demineralization and DuraPlex supplementation may attenuate this drop.

This research was completed in 2012. ✦

The Effect of Soaking or Steaming Timothy Hay on Voluntary Intake and Digestibility by Thoroughbreds

J.D. Pagan

Three mature Thoroughbred geldings were used in a 3 x 3 Latin square design trial to compare the intake and digestibility of dry timothy hay to the same hay that was either soaked or steamed. Voluntary intake, chewing time, and digestibility were recorded. During week 4, a complete collection trial was conducted and manure and hay samples were analyzed.

Voluntary intake was higher for the steamed hay than for the soaked or dry hay during the first week. Rate of intake was highest and number of chews rate was lower for the soaked hay. When expressed on a 100% dry matter basis, rate of intake and chewing rate were not different between treatments. Soaking or steaming reduces the water-soluble carbohydrate content of timothy hay, but it does not affect the digestibility of the protein, fiber, fat, ash, or residual sugar.

This research was conducted in 2012.✦

Effect of Water Dispersible Natural Vitamin E on Serum and Muscle Alpha-Tocopherol in Vitamin E Deficient Quarter Horses

J.D. Pagan

A supplementation study was conducted in five vitamin E deficient mature Quarter Horses.

For 4 weeks prior to the study and during the 6-week study period, horses were fed 1.5% of body weight in grass hay and 2 kg of sweet feed, and were exercised daily on a treadmill. The horses were supplemented with 5,000 IU of nanodispersed natural vitamin E (Nano-E®) in the morning feed 5 days a week. Serum and muscle samples were analyzed for alpha-tocopherol. Serum concentrations of vitamin E increased two-fold and muscle concentrations increased by over three-fold.

This study showed that high doses of water-dispersible natural vitamin E can increase alpha-tocopherol levels in muscles of deficient horses.

This research was published in *Proceedings of the Australasian Equine Science Symposium,* 2012. ✦

Fish Oil Supplementation Attenuates Abnormal Glucose Clearance Caused by High Dietary Fat Intake in Aged Thoroughbred Geldings

J.D. Pagan, B.M. Waldridge, J. Lange, C.G. Brown-Douglas, and P.J. Huntington

The aim of this study was to investigate the glucose dynamics in horses fed a high fat or moderate carbohydrate diet and to investigate if fish oil moderated these effects.

Four aged, nonobese Thoroughbred geldings were used in a 4 x 4 Latin square design study. Treatments consisted of grass hay, a vitamin/mineral supplement, and either oats and corn oil, oats and fish oil, hay cubes and soya oil and corn oil, or hay cubes and soya oil and fish oil. After four weeks on the diets, a frequently sampled glucose insulin test was performed. Plasma samples were tested for triglycerides, insulin, and glucose. Horses fed a high fat diet (hay cubes and corn and soya oil) had decreased glucose clearance compared with horses fed a moderate carbohydrate diet of oats and corn oil.

Fish oil significantly affected glucose clearance in the high fat diet but had no effect on glucose clearance in the moderate carbohydrate diet.

This research was published in P*roceedings of the Australasian Equine Science Symposium*, 2012. ✦

Moderate Dietary Carbohydrate Improved Glucose Tolerance and High Dietary Fat Impairs Glucose Tolerance in Aged Thoroughbred Geldings

J.D. Pagan, B.M. Waldridge, and J. Lange

This study was conducted to determine whether a moderate daily intake of carbohydrate from oats or a high level of fat intake from vegetable oil would affect glucose tolerance as measured by an intravenous glucose tolerance test.

A moderate intake of carbohydrate improved glucose clearance during the intravenous glucose tolerance test compared to n all-hay or high-fat diet. Blood glucose concentration returned to baseline in 126 ± 25.8 minutes with an oat-based diet (31% of digestible energy supplied by nonstructural carbohydrates) compared to 216.7 ± 23.5 minutes for the oil-based diet (30% of digestible energy supplied by soybean oil). The difference in return to baseline concentration was statistically significant.

The oat-based diet (31% of digestible energy supplied by nonstructural carbohydrates) is characteristic of most concentrate based feeds fed to horses. The alfalfa and soybean oil diet (30% of digestible energy supplied by soybean oil) had a much higher proportion of calories supplied by fat than typical equine rations. In other species, experimental diets high in fat have been used as an experimental model to produce glucose intolerance. This study demonstrated that a high-fat diet can produce the same effects in horses.

This research was published in *Proceedings of the American Association of Equine Practitioners*, 2011. ✦

Effect of Nonstructural Carbohydrate, Fat and Fiber Intake on Glycogen Repletion Following Intense Exercise

V.S. Mesquita, J.D. Pagan, S.J. Valberg, B.M. Waldridge, C. Whitehouse

Muscle glycogen is a potentially limiting substrate for horses during intense exercise. Low-starch, high-fat concentrates have become popular for performance horses, but their effect on muscle glycogen usage and repletion is unclear.

Six Thoroughbred horses were studied in a 3x3 Latin Square design to measure the effect of non-structural carbohydrate (NSC), fat and fiber intake on glycogen repletion following intense exercise. Horses were fed isocaloric, isonitrogenous rations that supplied high (HS), medium (MS) or low (LS) NSC intake. The horses were fed 1.25% BW/d grass-hay and 1.0% BW/d concentrate. These rations provided 45%, 36% and 18% of total digestible energy (DE) from NSC, 11%, 15% and 23% of total DE from fat, and 30%, 33% and 45% of total DE from fiber. During each 1-month period horses were trained for three weeks on a high-speed treadmill followed by a three-day glycogen-depletion period comprising multiple bouts of intense exercise. Muscle biopsies were taken before and 0, 24, 48 and 72 hours post-depletion. A standardized exercise test (SET) was performed on day 1 of the depletion period.

During the SET, lactate was lower in LS compared to MS or HS ($P<0.05$). Heart rate, plasma glucose, VO_2, VCO_2 were unaffected by treatment. Muscle glycogen depletion averaged 33%, 30%, and 36% in the HS, MS and LS treatments. Muscle glycogen repletion was significantly lower in the LS group 72 hours post exercise ($P<0.05$).

Low NSC concentrates may not provide enough substrate for glycogen repletion following multiple bouts of intense exercise.

This research was published in *Proceedings of the 24th Equine Science Society Symposium,* 2015. ✦

Furosemide Administration Affects Mineral Excretion in Exercised Thoroughbreds

J.D. Pagan, B. Waldridge, C. Whitehouse, S. Fuchs, M. Goff

Furosemide is widely administered to Thoroughbred and Standardbred racehorses to reduce the incidence and severity of EIPH. The magnitude and duration of mineral loss following furosemide administration has not previously been measured in horses.

Six fit Thoroughbreds (age 6.8 ± 1.2 y; bwt 585 ± 19 kg) were used in a 3x3 Latin square design trial to measure the effect of furosemide on urinary and fecal mineral excretion for 24 h before (−24 h) and 24 h, 48 h and 72 h after a standardized exercise test (SET) on an inclined (3º) high speed treadmill. The treatment groups consisted of an untreated control (C) and two groups (F and FE) which received furosemide (0.5 mg/kg, IV) 4 h before the SET. C and F were fed 60 g/d NaCl and (FE) was fed 200 g/d of an electrolyte mix (14 g K, 37.7 g Na and 43.3 g Cl) for 21 days before the SET. Following the SET the FE group was supplemented with an additional 10 g/d Ca and 10 g/d Mg.

Urinary Ca, P, Na and Cl excretion increased 24 h post treatment in the F and FE groups ($P<0.05$). 72 h Ca and Cl balance was decreased in F ($P<0.05$) and 72 h Mg and Cl balance was decreased in FE ($P<0.05$). The digestibility of P and Mg was decreased in FE ($P<0.05$) and Na digestibility was increased in F and FE ($P<0.05$).

A single dose of furosemide negatively affected calcium balance in horses for 72 h post administration. Further research is needed to assess the effect of chronic furosemide use in racehorses on mineral balance and soundness.

This research was published in *Proceedings of the 23rd Equine Science Society Symposium,* 2013.✦

Dextrose Does Not Affect Rate of Absorption or Retention of Electrolytes in Idle Thoroughbreds

J.D. Pagan, B.M. Waldridge, and J. Lange

Two studies were conducted to evaluate the effects of dextrose or corn starch on uptake and retention of electrolytes and water in idle horses.

In study 1, four Thoroughbreds (age: 6.25 ± 2.25 y; BW: 574.4 ± 82.4 kg) were used in a 4 X 4 Latin square trial. The horses were dosed with 92 grams of electrolyte (72 g NaCl, 20 g KCl) either alone (elect), with 10 g of dextrose, or 100 g dextrose. The electrolyte mixes were dissolved in 1 liter of water and administered via nasogastric tube. A fourth treatment of 1 liter water with no added electrolytes or dextrose served as a control. Plasma samples were taken before and for 4 h post dosing, and Na+, K+, Cl−, BUN, and glucose were measured. The horses were offered water free choice, and hourly water intake was measured for 4 h post dosing. Plasma Na+ and osmolality were significantly elevated post dosing in all three electrolyte treatments compared to the control ($p<.05$), but dextrose did not affect the rate or duration of increase. All electrolyte treatments increased voluntary water intake for the first 4 h post dosing compared to the control ($p<.05$). Water intake equaled 0.3 ± .4 l, 5.3 ± 3.6 l, 5.4 ± 2.3 l, and 4.7 ± 2.5 l in the control, elect, 10 g dex, and 100 g dex treatments, respectively.

A second 4 X 4 Latin square trial was conducted with four Thoroughbreds (age: 6.25 ± 2.25 y; BW: 546.6 ± 35.2 kg). The horses were administered 1 mL distilled H2O/100 g BW + 0.15 g/kg BW D2O via nasogastric tube either (1) alone (control), (2) with 70 g NaCl + 30 g KCl (elect), (3) electrolyte + 10 g dextrose (dex), or (4) electrolyte + 10 g corn starch (starch). Blood samples were taken immediately before and .5, 1, 2, 3, and 4 h post dosing, and D2O, Na+, K+, Cl−, BUN, and glucose were measured. Plasma D2O at 2 h post dosing was used to calculate total body water. Total urine and fecal excretion was measured for 24 h before dosing and at 12-h intervals for 72 h post dosing. Plasma Na+ and osmolality were significantly elevated post dosing in all three electrolyte treatments compared to the control ($p<.05$), but neither dextrose nor starch affected the rate or duration of increase. Plasma D2O was elevated to a greater extent ($p<.05$) in the control compared to the three electrolyte treatments at 30 and 60 min post dosing, suggesting that isotonic electrolyte solutions delay water uptake compared to pure water. Total body water was unaffected by treatment and equaled 59.1 ± 6.3, 60.3 ± 5.3, 62.6 ± 7.7 and 58.4 ± 4.7 ml/kg BW for the control, elect, dex, and starch treatments, respectively. Urinary and fecal electrolyte excretion was not different between the 3 electrolyte treatments.

This research was published in the *Proceedings of the 23rd Equine Science Society Symposium*, 2013. ✦

The Effect of Soaking or Steaming Timothy Hay on Voluntary Intake and Digestibility by Thoroughbreds

J.D. Pagan, C. Whitehouse, A.M. Grev, S.W. Garling, and O.L. Yates

Three mature Thoroughbred geldings were used in a 3 x 3 Latin square trial to compare the intake and digestibility of dry timothy hay (DRY) to the same hay that was either soaked (SOAKED) or steamed (STEAMED) in a Haygain hay steamer.

Each period lasted four weeks. The voluntary intake (kg/day), rate of intake (grams consumed/minute), chewing rate (number of chews/100 g), and apparent digestibility of dry, soaked, and steamed mature timothy hay were investigated. The STEAMED hay was placed in a half-bale HAYGAIN steamer and allowed to reach a temperature of 1700 C. SOAKED hay was submerged for 1 hour in 18 gallons (~68 l) of ambient temperature water and allowed to drain for 30 minutes in hay nets before feeding. During week 1, voluntary daily hay intake (air dry hay equivalent) averaged 13.8 ± .3, 12.5 ± .5, and 14.2 ± .2 kg (mean ± SEM) for the DRY, SOAKED, and STEAMED hay, respectively. These intakes equaled 2.1%, 1.9%, and 2.2% of BW/day. STEAMED intake was significantly higher than SOAKED ($p<.05$). Rate of intake on an as-fed basis was significantly faster for the SOAKED hay compared to the DRY and STEAMED ($p<.05$). Conversely, the number of chews/100 g of as-fed intake was significantly lower for the SOAKED compared to DRY and STEAMED ($p<.05$). When expressed on a 100% DM basis, rate of intake and chewing rate were not different between treatments. The fiber components (ADF and NDF) of the SOAKED and STEAMED hays were greater than the DRY hay. This was due to a loss of other components from the SOAKED and STEAMED hays. If these compositions were recalculated, assuming that the NDF content of the hays were equal, then about 9% of the original dry matter of the SOAKED hay and 4% of the STEAMED hay dry matter were lost as a result of soaking and steaming. Much of this loss was from WSC. Soaking or steaming did not affect apparent nutrient digestibility.

Soaking or steaming reduced the WSC content of timothy hay, but it did not affect the digestibility of the protein, fiber, fat, ash, or residual sugar. Steaming increased the free-choice intake of hay compared to soaking, but rate of intake and the amount of chewing was not affected when expressed on a dry matter basis.

This research was published in the *Proceedings of the 23rd Equine Science Society Symposium*, 2013. ✦

Furosemide Reduces the Energetic Cost of Exercise in Thoroughbreds Independent of Its Effect on EIPH

J.D. Pagan, B.M. Waldridge, C. Whitehouse, L. Dalglish, S. Fuchs, and M. Goff

Six fit Thoroughbred geldings (age: 6.8 ± 1.2 y, BW: 585 ± 19 kg) were used in a 3 X 3 Latin square trial to test the effect of furosemide administration on energetic efficiency during a standardized exercise test (SET) on an inclined (30) high-speed treadmill.

The SET consisted of 5 min at 1.7 m/s, 2 min at 4 m/s, 6 m/s, and 8 m/s, and 1 min at 9 m/s, 10 m/s, 11 m/s, 12 m/s, and 13 m/s. The horses were not allowed access to water, hay, or feed for 4 h before the SET. The treatment groups consisted of a control (C), which received no furosemide before the SET, and two groups (F and FE), which received furosemide IV (.5 mg/kg BW) 4 h before the SET. C and F were fed 60 g/d NaCl and (FE) was fed 200 g/d of an electrolyte mix which that provided 14 g/d K^+, 37.7 g/d Na^+, and 43.3 g/d Cl^- for 21 days before the SET along with 5.85 kg hay, 4.0 kg grain and .12 kg of a vitamin/mineral supplement. The horses were weighed 4 h before the SET (before furosemide administration), immediately before and after the SET, and 4 h, 8 h, 12 h, 24 h, 48 h, and 72 h post exercise. Venous blood samples were taken from the horses -4 h, -3 h, and immediately before the SET; at the end of each speed throughout the SET; and 5 min, 1 h, and 4 h post exercise. Blood was analyzed for lactate, glucose, total protein (TP), and packed cell volume (PCV). Heart rate (HR), VO2, and VCO2 were measured during the last 30 s of each speed during the SET. An endoscopic tracheobronchial assessment was conducted 1 h post exercise and the degree of EIPH graded.

Weight loss during the 4-h period before the SET was higher in F (12.0 ± 1.5 kg) and FE (12.7 ± 0.84 kg) compared to C (6.67 ± 1.2 kg) ($p<.05$). During the SET, HR was higher in C compared to either F or FE ($p<.05$). Absolute VO2 (l/min) and VCO2 (l/min) were higher in C compared to F and FE ($p<.05$). When expressed on a weight- adjusted basis (ml/kg BW/min), VO2 and VCO2 were not different between treatments. Lactate accumulation was higher in C compared to F ($p<.05$). TP was higher in F and FE compared to C ($p<.05$), and PCV was higher in FE compared to C ($p<.05$). The incidence of EIPH was very low and unrelated to treatment.

The energy cost of exercise is the sum of energy generation from aerobic and anaerobic pathways, which can be estimated from oxygen consumption and lactic acid accumulation. Furosemide administration reduced energy generation from both pathways during exercise and this improvement was probably due to a reduction in body weight.

This research was published in the *Proceedings of the 23rd Equine Science Society Symposium*, 2013. ✦

Furosemide Administration Affects Mineral Excretion in Exercised and Non-Exercised Thoroughbreds

J.D. Pagan, B.M. Waldridge, C. Whitehouse, L. Dalglish, S. Fuchs, and M. Goff

Two studies were conducted to measure the effect of furosemide on 72-h digestibility and urinary excretion of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), and chloride (Cl) in non-exercised and exercised mature Thoroughbred geldings.

In study 1, total fecal and urinary mineral excretion was measured in three unexercised Thoroughbred geldings for 72 h before and 24 h, 48 h, and 72 h after a single dose (.5 mg/kg IV) of furosemide. The horses were fed 8.3 ± 0.98 kg orchard grass hay, 1 kg of grain mix, and 120 g of a vitamin/mineral supplement daily for 15 days before and during the 6-day collection period. Urinary sodium excretion increased 24 h post furosemide administration but returned to pretreatment levels 48-72 h post treatment. Urinary Ca, K, and Cl excretion increased post treatment and remained elevated 72 h post treatment. 72-h Ca, K, and Cl balances were lower post treatment than pretreatment ($p<.05$). Urinary P and Mg excretion was unaffected by treatment. Digestibility of all minerals was unaffected by treatment.

In study 2, six fit Thoroughbred geldings (age: 6.8 ± 1.2 y, BW: 585 ± 19 kg) were used in a 3 X 3 Latin square trial to measure the effect of furosemide on urinary and fecal mineral excretion for 24 h before (-24 h) and 24 h, 48 h, and 72 h after a standardized exercise test (SET) on an inclined (3º) high-speed treadmill. The SET was performed at 1.7 m/s for 5 min; 4 m/s, 6 m/s, and 8 m/s for 2 min per step; and 9 m/s, 10 m/s, 11 m/s, 12 m/s, and 13 m/s for 1 min per step. The horses were not allowed access to water, hay, or feed for 4 h before the SET. The treatment groups consisted of an untreated control (C) and two groups (F and FE), which received furosemide (0.5 mg/kg, IV) 4 h before the SET. C and F were fed 60 g/d NaCl, and (FE) was fed 200 g/d of an electrolyte mix (14 g K^+, 37.7 g Na^+, and 43.3 g/d Cl^-) for 21 days before the SET along with 5.85 kg hay, 4 kg grain, and .12 kg of a vitamin/mineral supplement. Following the SET, the FE group was supplemented with an additional 10 g/d Ca and 10 g/d Mg. Urinary Ca, P, Na, and Cl excretion increased 24 h post treatment in the F and FE groups ($p<.05$) compared to −24 h and were higher than C 24 h levels ($p<.05$).

This research was published in the *Proceedings of the 23rd Equine Science Society Symposium*, 2013. ✦

Evaluation of Apparent Total-Tract Digestibility and Glycemic Responses to Processed Corn in Nonexercised Thoroughbred Horses

C. Whitehouse, J.D. Pagan, R.J. Coleman, B. Waldridge, O.L. Yates, and S.W. Garling

In the process of extracting starch from corn for food manufacturing, the co-product corn germ dehydrated (AAFCO 48.32) is produced. This fraction is known commercially as Golden Max™ (GM).

A study was conducted to investigate the nutritional value of GM for horses and compare it to the typical forms of processed corn commonly fed to horses, cracked (CC) and steam-flaked corn (SF). Diets consisted of a control (no corn) and 3 processed corn diets fed with added fiber sources. The four diets were formulated to supply similar intakes of neutral detergent fiber (NDF) and acid detergent fiber (ADF) from a combination of corn bran, alfalfa cubes, and alfalfa meal. CC, SF, and GM diets were fed to provide a starch intake of 6 g/kg BW/d, offered in 3 equal meals.

Four mature Thoroughbred geldings with an average body weight of 552 ± 10 kg were used in a 4×4 Latin square digestibility trial. Each experimental period was 21 d made up of 5 d adaptation, 11 d on treatment diet, and 5 d total fecal collection. Fecal pH was measured on the last day of each period. The area under the glucose-concentration time curve was used to categorize the pre-cecal starch digestibility of GM, CC, and SF based on 1 kg corn and starch intake per meal (1 g starch/kg BW). Plasma samples were taken pre-feeding and every 30 min post feeding for 240 min and analyzed for glucose.

Apparent total-tract digestibilities (mean % ± SE) were determined for dry matter (DM), crude protein (CP), starch, ADF, NDF and gross energy (GE). All 3 corn diets had significantly higher DM digestibility than the control ($P < 0.05$). CC had lower CP digestibility ($P < 0.05$). NDF and ADF digestibility coefficients were not different between diets ($P > 0.05$). Total-tract starch digestibility was high in all diets ranging from 92 ± 5 for the control, 97 ± 1 for CC, 99 ± 0.4 for SF, and 99 ± 0.4 for GM. Mean DE (Mcal/kg DM) values for CC, SF, and GM estimated by difference from the control diet were 3.22 ± 0.12, 3.84 ± 0.11, and 3.87 ± 0.06, respectively. DE in CC was lower than GM or SF ($P < 0.05$). Horses fed

1 kg of SF had a larger glycemic response than GM and CC ($P < 0.05$). On an equal starch-comparison, there was no difference in response between CC, SF, and GM ($P = 0.12$). Horses fed CC had a lower fecal pH than control and GM ($P < 0.05$). GM contained highly digestible starch that did not alter fecal pH even when fed at high levels of intake. Compared to SF, GM contained less starch (54.9% vs 73.8%), higher fat (9.8% vs 3.1%), and similar DE content.

GM is a suitable alternative source of carbohydrate and fat compared to traditionally used processed corns.

This research was published in *Proceedings of the 24th Equine Science Society Symposium*, 2015. ✦

Intensity of Exercise During Early-Season Competition in Three-Day-Event Horses Assessed Using KER ClockIt™ Sport Smartphone Application

J.D. Pagan, K. O'Neill, N. Ireland, and M. Davies

Training protocols should be designed taking into account the intensity and duration of exercise during competition. This study measured heart rate (HR), duration, speed, and distance traveled during the cross-country phase of 32 competitions held in the early stages of the 2015 American eventing season using a novel smartphone application (KER ClockIt™ Sport; Kentucky Equine Research, Versailles, KY).

A Bluetooth-equipped heart-rate monitor (Polar H7) was used to measure and transmit HR data to the application. Twenty-eight horses competing at four different competition levels participated in this study. All horses were trained in Ocala, Florida; Aiken, South Carolina; or Southern Pines, North Carolina, and they competed at six different venues in Georgia, Florida, and North Carolina. The competitions were held in late February, March, and early April 2015. Exercise intensity was defined based on specific HR zones expressed as a percent of maximal HR (HRmax), which was assumed to be 220 BPM. Measurements were collected for the entire cross-country phase of each three-day event, including the warm-up period. Horses typically warmed up for approximately 30–45 min.

Horses competing at Training, Preliminary, Intermediate, and Advanced levels spent an average of 3.3, 4.5, 2.9, and 3.6 minutes in HR zone 5 (175–200 bpm), respectively. Horses competing at the Intermediate and Advanced levels spent an average of 2.8 and 4.3 minutes with HR greater than 200 (zone 6). Advanced horses spent significantly more time in HR zone 6 than either the Training or Preliminary horses. There was no significant difference in how much time horses at each level spent in HR zones 4 and 5. During the cross-country phase, horses competing at all levels spent several minutes at HRs greater than 80% of HRmax, and Intermediate and Advanced horses spent a significant amount of time with HR > 90% HRmax.

These data suggest that training protocols for all levels of event horses should contain exercise intensities in these higher HR zones.

This research was published in the *Proceedings of the 25th Equine Science Society Symposium*, 2017. ✦

Estimating Digestible Energy Requirements of Three-Day-Event Horses Using KER ClockIt™ Sport Smartphone Application

J.D. Pagan, K. O'Neill, N. Ireland, and M. Davies

This study estimated digestible energy (DE) requirements of three-day-event horses during training in the early stages of the 2015 American eventing season using a novel smartphone application (KER ClockIt™ Sport; Kentucky Equine Research, Versailles, KY).

Heart rate (HR), session duration, and distance traveled were measured in 26 eventers representing 5 different levels of competition. The horses included 5 Advanced (including 3* and 4*), 5 Intermediate (including 2*), 7 Preliminary (including 1*), 5 Training, and 4 Novice level horses of Thoroughbred, Warmblood, and other sport-horse bloodlines. A Bluetooth-equipped heart-rate monitor (Polar H7; Polar Electro Inc., Lake Success, NY) was used to measure and transmit HR data to the application.

Velocities were divided into 5 ranges corresponding to the most common gait at each speed. During each training session, time and average HR at each gait were calculated. These data were used to calculate energy expenditure using a regression equation that estimated oxygen consumption from HR. Weekly energy expenditures were calculated for each horse, and DE requirements were estimated based on maintenance DE requirements and previously determined efficiencies of utilization of DE for exercise. DE requirements were expressed as percentages of maintenance DE. DE requirements for horses training at Novice, Training, Preliminary, Intermediate, and Advanced levels averaged 124%, 131%, 145%, 158%, and 148% of maintenance, respectively.

DE requirements (% maintenance) were highly correlated with both total distance (km) trained per week (DE % = 1.337 X km traveled/wk) + 96.7)($R2=.98$) and total time (hr) spent training per week (DE % = 13.46 X hr exercise/wk) + 86.0)($R2=.95$). HR and duration of exercise can be used to estimate DE requirements for different disciplines. These equations can then be used to estimate DE requirements for individual horses by monitoring their weekly activity with the KER ClockIt™ Sport smartphone application.

This research was published in the P*roceedings of the 25th Equine Science Society Symposium,* 2017. ✦

Intensity and Distance of Exercise During Training in Advanced Three-Day-Event Horses and Thoroughbred Racehorses Assessed Using KER ClockIt™ Race Smartphone Applications

J.D. Pagan, E. Mulvey, K. O'Neill, N. Ireland, and M. Davies

Nutrient requirements for performance horses are dependent on the intensity and duration of exercise during training and competition. Differences between racehorses and upper-level event horses have not been previously defined. This study measured daily heart rate (HR), session duration, and distance traveled in 11 Advanced-level three-day-event horses and 6 Thoroughbred racehorses during training over a 2-month period using novel smartphone applications (KER ClockIt™ Sport and KER ClockIt™ Race; Kentucky Equine Research, Versailles, KY and ClockItEQ, Perth, WA).

A Bluetooth-equipped heart-rate monitor (Polar H7) was used to measure and transmit HR data to the application. Velocities were divided into 5 ranges corresponding to the most common gait at each speed. Training intensity was defined based on specific HR zones expressed as a percent of maximal HR (HRmax), which was assumed to be 220 BPM.

The event horses were in training in Ocala, Florida, during February and March 2015, while the racehorses were in training in Lexington, Kentucky, during July and August 2016. The event horses participated in a total of 33 competitions and the racehorses raced a total of 11 times during the study. Racehorses were typically ridden 6 days a week and hand-walked once a week for 15 minutes. Training exercise sessions consisted of either galloping, jogging, or 600-1000 m breezes and averaged 20 minutes in duration. After an exercise bout, racehorses typically hand-walked for 20 minutes and hand-grazed for 15 minutes. Event horses did a wide variety of exercise including hacking, trot sets, jumping, cross-country schooling, and gallop sets. These exercise sessions averaged 40-45 minutes in duration.

The intensity and duration of training was different between event horse and racehorses. Event horses trained over twice the weekly distance compared to racehorses (37.2 km vs 17.1 km), but their intensity of training was lower. Racehorses galloped an average of 2.7

days/wk compared to event horses, which only galloped 0.5 days/wk. During each gallop session, racehorses galloped further (2130 m vs 520 m) and spent more time with HR >80% HRmax (2.5 min vs 0.5 min) than event horses.

This research was published in the *Proceedings of the 25th Equine Science Society Symposium,* 2017.✦

Comparison of Processed and Unprocessed Digital Images for the Determination of Radiographic Bone Aluminum Equivalent (RBAE) Values in Equine Bone

J.D. Pagan, P. Kazakevicius, A. Swanhall, E. Ford, and A. Pritchard

Radiographic photodensitometry to estimate bone density in horses was originally developed using conventional analog film radiography. Today, most researchers have switched to digital radiography (DR) to make these measurements. One important feature of DR is that the raw data is processed after acquisition. Processing algorithms are generally proprietary and specific to the DR vendor, but they encompass manipulations such as adjustment of contrast curves and application of nonlinear image filters to optimize image quality parameters such as contrast and noise. These processing steps have a profound effect on the final appearance of the radiograph, and they can also lead to artifacts unique to digital systems that may affect density measurements.

This study measured radiographic bone aluminum equivalent (RBAE) values in the left third metacarpal (MCIII) of 16 Thoroughbred horses (3.2 ± 0.4 y) using raw (unprocessed) and processed images acquired using a portable X-ray unit and read by a DR system (Sound NEXT Equine DR).

Radiographs of the dorsal-palmar and lateral-medial views of MCIII were taken. An aluminum step wedge placed in the same plane as the MCIII was exposed simultaneously to calculate RBAE values. Unprocessed and processed images were analyzed using ImageJ image-processing software. An optical density calibration curve was created for each image using the known thicknesses of the step wedge. This relationship was best described using a natural log equation in the unprocessed images and a 4th degree polynomial equation in the processed images. Image processing had a large, inconsistent effect on RBAE values. Peak densities were 32-68% higher in the processed images and many were above the density of the thickest step of the step wedge. In lower density areas, the processed images had lower densities than the unprocessed images, demonstrating the nonlinear nature of the processing algorithm. RBAE values obtained from unprocessed images were similar to values measured in previous Kentucky Equine Research studies using analog film radiology. Raw (unprocessed) images should be used with DR systems to determine RBAE in equine bone.

This research was published in the *Proceedings of the 25th Equine Science Society Symposium,* 2017. ✦

Water, Concentrate, and Hay Intake in Thoroughbred Racehorses

J.D. Pagan, A. Haas, A. Swanhall, E. Ford, E. Rugg, A. Bouquet, and A. Pritchard

Water, hay, and feed intake was measured in 6 Thoroughbred racehorses (age 3.3 ± 0.5 y) (BW 468 ± 14.1 kg) for 56 d at a training center in Lexington, Kentucky, during July and August 2016. Horses were kept in stalls in a typical race-training environment and offered free-choice timothy hay. A concentrate formulated for racehorses was fed in 3 meals/d. Each morning, hay was weighed prior to feeding and placed in a ground feeder. After 24h, the remaining hay in the feeder was removed and weighed to determine consumption. Water intake was measured three times per day.

Racehorses were typically ridden 6 days a week and hand-walked once a week for 15 minutes. Training consisted of either galloping, jogging, or 600-1000 m breezes. Horses galloped 1600-2400 m an average of 2.7 times per week. Exercise sessions averaged 20 minutes. After an exercise bout, racehorses typically hand-walked for 20 minutes and hand-grazed for 15 minutes. During the study, the horses either raced or breezed weekly except for the week after a race when the horses usually only walked and jogged. Horses raced a total of 11 times during the study. Because of logistics associated with racing, hay and water intake was not measured on race days. All horses received furosemide before racing.

Water intake averaged 30.3 l/d and was higher the day after racing and on gallop days than when the horses were hand-walked. There was a positive relationship between average daily temperature, which ranged from 21-28o C, and water intake. Water intake increased 4-5% for each 1o C increase in temperature. Free-choice hay intake averaged 4.4 kg/d and was unaffected by work type, temperature, or water intake. Hay intake averaged 1% BW, which was lower than expected, but was similar to intakes reported for racehorses in Australia. Concentrate intake equaled 7.7 ± 0.6 kg/d. Horses maintained body weight throughout the study. Digestible energy intakes averaged 31.5 ± 0.9 Mcal/d, which is in agreement with 2007 NRC recommendations for racehorses.

This research was published in the *Proceedings of the 25th Equine Science Society Symposium*, 2017. ✦

Notes

Nutrient Content of Australian Horse Pastures—Effect of Season

P.J. Huntington, C.G. Brown-Douglas, and L. Wood

Most Australian horses rely on pasture for some of their nutrient intake, and many are just on pasture without supplementary feed for some part of the year. To determine the nutrient intake of horses on pasture for comparison with recommended daily allowances, nutritionists need to know not only the pasture intake but also the nutrient content of the pasture. However, pasture analysis is uncommon, so nutritionists must rely on estimates of nutrient composition.

A total of 435 samples from pastures grazed by horses in all states from 2000 to 2014 were submitted to Equi-Analytical Laboratories, Ithaca, New York, for forage NIR analysis. Some pastures were sampled several times during different seasons and years, and multiple pastures were sometimes sampled from the one property. Most were improved pastures in temperate regions with an emphasis on Thoroughbred studs. Some pastures were kikuyu-dominated, and some contained significant lucerne content, but specific pasture composition details were not recorded routinely. Some were irrigated, but most were not. These results were tabulated and calculations made for average, standard error, normal range, mineral ratio, and effect of month and season. Statistical analysis used Tukey-Kramer ANOVA tests set at $p < 0.05\%$.

Table 1: Seasonal difference in pasture composition. Mean and superscripts indicating significant difference ($P<0.05$) from other seasons: a = summer, b= autumn, c= winter, d= spring.

Season	Summer	Autumn	Winter	Spring
No. of Samples	87	81	129	138
Dry Matter, %	28.6^{bcd}	25.0^{ac}	21.6^{abd}	24.4^{ac}
DE, (Mcal/kg)	1.93^{cd}	1.96^{cd}	2.11^{ab}	2.12^{ab}
Crude Protein, %	16.1^{cd}	18.3^{c}	22.9^{abd}	18.7^{ac}
NDF, %	62.0^{cd}	58.7^{c}	51.3^{ab}	55.9^{ab}
WSC, %	5.2^{cd}	5.3^{cd}	7.8^{ab}	7.8^{ab}
Ca, %	0.54^{b}	0.64ad	0.62a	0.54b
P, %	0.32^{bcd}	0.38^{a}	0.41^{a}	0.37a
Zn, ppm	32.9	33.7	33.8^{d}	29.9^{c}
Cu, ppm	8.3	8.7	8.0	8.2
Quarter	1 (Jan, Feb, Mar)	2 (Apr, May, Jun)	3 (Jul, Aug, Sept)	4 (Oct, Nov, Dec)
Ca:P ratio	1.89^{ac}	2.32^{acd}	1.39^{ab}	1.7^{b}

There were significant seasonal differences in many nutrients as shown in Table 1. Interestingly, the winter pastures had the highest protein content whilst energy and WSC content was higher in winter and spring than summer and autumn. Not surprisingly, fibre content was highest in summer and dry matter lowest in winter. There were seasonal differences in calcium (Ca) and phosphorus (P) content, but little difference in the trace minerals zinc and copper. Seasonal changes in Ca and P content did not reflect the influence on the vital Ca:P ratio in horse pastures. The Ca:P ratio was best examined by quarter rather than season. In Q3, the mean Ca:P ratio of 1.39 was lower than in Q1 and Q2. Importantly only 11% of pastures had a Ca:P content less than 1:1 in Q1 and Q2, but 25% pastures were affected in Q3 and 16% in Q4. This situation would create problems with calcium resorption from bone in horses not receiving supplementary feed, and is more widespread than was previously recognised in late winter and early spring. Pasture analysis would be needed to detect the problem and should be performed more commonly to better define the contribution pasture can make to horse nutrition and the needs of supplementary feeding.

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Nutrient Content of Australian Horse Pastures—Nutrient Intakes Compared to Requirements

P.J. Huntington, C.G. Brown-Douglas, and L. Wood

Most Australian horses rely on pasture for some of their nutrient intake, and many are just on pasture without supplementary feed for some part of the year. To determine the nutrient intake of horses on pasture for comparison with recommended daily allowances, nutritionists need to know not only the pasture intake but also the nutrient content of the pasture. However, pasture analysis is uncommon, so nutritionists must rely on estimates of nutrient composition.

A total of 435 samples from pastures grazed by horses in all states from 2000 to 2014 were submitted to Equi-Analytical Laboratories, Ithaca, New York, for forage NIR analysis. Some pastures were sampled several times during different seasons and years, and multiple pastures were sometimes sampled from the one property. Most were improved pastures in temperate regions with an emphasis on Thoroughbred studs. Some pastures were kikuyu-dominated, and some contained significant lucerne content, but specific pasture composition details were not recorded routinely. These results were tabulated and calculations made for average, range, standard deviation, normal range (+/- 1 SD), estimated fructan content (WSC-ESC), mineral ratio, and effect of season. Statistical analysis used ANOVA with significance set at $p<0.05$. Average values were used to compare nutrient intakes and requirements for different classes of horse grazing pasture alone without any supplementary feed. It was assumed that horses would eat 100% NRC (2007) dry matter intakes.

Table 1. Results of average and normal range (+/- 1 SD) from nutrient analysis of 435 horse pastures

	DM %	DE Mcal/kg	CP %	ADF %	NDF %	WSC %	Fructan %	NFC %	Cr Fat %	Ash %
Ave	25.3	2.05	19.3	34.7	56.3	6.9	1.95	11.3	3.4	11.3
Lower	14.8	1.84	12.8	28.9	46.8	3.8	2.2	6.0	2.3	8.8
Upper	35.8	2.27	25.9	40.6	65.7	9.9	3.5	16.6	4.6	13.7
	Ca %	P %	Ca:P	Mg %	K %	Na %	Fe ppm	Zn ppm	Cu ppm	Mn ppm
Ave	0.60	0.38	1.8	0.27	2.97	0.25	208	33	8.3	87
Lower	0.33	0.24	0.64	0.18	1.92	0.05	65	17.5	5.4	19
Upper	0.86	0.52	2.98	0.36	4.02	0.44	350	47.8	11.2	156

The pastures samples had lower DM, DE, WSC, ESC, NFC, and Fe content, and higher Na, than the mixed-mostly-grass pasture in the Equi-Analytical Laboratory feed library, which is drawn from over 11,000 samples. A 500-kg horse at maintenance grazing the average pasture would have deficient copper and zinc intakes in all seasons and would have excess DE intake in winter and spring, allowing it to gain weight. The same horse in the "lower normal" pasture would be deficient in Ca, Na, Cu, Zn, and Mn. Using seasonal average figures, a lactating Thoroughbred mare would have a diet that was underfortified for Cu in all seasons and would have excessive DE intake in winter and spring so could gain weight. A late-pregnant mare on pasture alone would have a diet that was underfortified for Cu and Zn. A 250-kg, 6-month-old Thoroughbred weanling growing at 0.9 kg/d would have deficiencies of DE, Ca, P, Cu, and Zn grazing the average summer pasture, and DE, Cu, and Zn on the average autumn pasture. The expected growth rate could not be achieved without supplementary energy sources. A 350-kg, 12-month-old yearling growing at 0.6kg/d would be deficient in DE, Ca, Cu, and Zn on average spring pasture.

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Mineral and Vitamin Supplementation Including Marine-Derived Calcium Increases Bone Density in Thoroughbreds

J.D. Pagan, A. Swanhall, E. Ford, E. Mulvey, and P.J. Huntington

Bone quality is important for the long-term health and soundness of performance horses. In racehorses, insufficient bone density is associated with shin soreness, an important cause of lost training time and reduced starts, as well as other aspects of bone fatigue. Risks of injury during training are associated with insufficient bone density. Research has previously identified several minerals and vitamins with an influence on bone and positive effects on bone density. Marine-derived calcium mineral complexes have been shown to improve bone density and strength in laboratory animals and humans. This study evaluated the effect of a supplement containing marine-derived calcium (TriactonTM) (TA) on bone density in Thoroughbred racehorses.

Thirteen Thoroughbred racehorses in race training were studied for 90 days. The average age of the horses was 3.1 ± 0.4 years. Horses consisted of two- and three-year-olds that were beginning race training, three-year-olds which had been in active race training, and older horses returning to training after a 60- to 90-day break from active training. Horses were fed timothy hay and a fortified concentrate at levels required to maintain body weight throughout the study. The base diet supplied 64 g Ca and 42 g P/d. Seven horses received 120 g/d of TA, which supplied an additional 15 g Ca and 3g P, and 7 horses received 120 g/day of a placebo pellet (CON). The supplement contained calcium, phosphorus, magnesium, silicon, boron, copper, zinc, manganese, and vitamins A, D, K, and C. The horses were paired by age and exercise intensity and then randomly assigned to treatments. One horse from the placebo group was removed from the study because of lameness unrelated to bone development and has not been included in study results. There was no difference in average age between the two groups. Training consisted of slow and fast work. The average total distance trained by the horses at different gaits in each treatment group was measured and there was no difference in training intensity or duration between the two treatment groups.

Digital radiographs of the left fore metacarpus were taken from dorsal-palmar and lateral-medial views at 0, 4, 8, and 12 weeks of training. An aluminium step-wedge was placed in plane with the metacarpus to use RBAE as an external measure of bone density. Radiographic photodensitometry was used to measure the density of the lateral, medial, dorsal and palmar cortices of the metacarpus were measured 1cm below the nutrient foramen. The thickness of each cortex and the overall thickness and width of the metacarpus were measured. Differences due to training duration and supplementation were determined using a 2-way ANOVA.

Over the 12-week training period, the horses supplemented with TA increased dorsal and palmar cortical bone density ($p<.05$). CON densities were not affected by training. Medial and lateral cortical bone densities were not affected by treatment nor was cortical thickness or overall width.

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Effect of Omeprazole and Calcium Sources on Calcium Digestibility in Thoroughbred Horses

J.D. Pagan, L.A. Petroski, A.C. Mann, A.A. Hauss, and P.J. Huntington

Equine gastric ulcer syndrome (EGUS) is very common, with a prevalence estimated from 53% to 93%. A major contributor to its pathogenesis is excessive gastric acid. Omeprazole (OM) is a proton pump inhibitor (PPI) that inhibits gastric acid secretion in horses and is the most popular treatment for EGUS. PPIs are also widely used in humans to treat acid-related conditions and have been associated with a reduction in the digestibility of several nutrients including protein, fat, calcium (Ca), and iron. Epidemiological studies have also found a positive association between PPI and the risk of osteoporotic fractures. It is not known how a therapeutic dose of OM affects nutrient digestibility or bone metabolism in horses. This study was conducted to determine the effect of administration of omeprazole on the digestibility of diets containing two calcium sources and to assess changes in blood parameters associated with gastric acid production and Ca status.

Four mature Thoroughbred geldings (age 11.25 ±4.11 y; BW 585.4±25.4 kg) were used in a 2 x 2 factorial design to evaluate the digestibility of diets containing two different Ca sources, limestone (CC) or a marine-derived Ca source (BMC with or without OM) over 4 x 21d periods. Treatments were assigned so that OM was not given to a horse in two sequential periods. Each 21d period had a 13-d diet adaptation phase followed by an 8-d collection phase, which consisted of a 48-h adaptation period to collection harnesses, a 5-d total fecal collection period and a final day for gastroscopy and blood sampling. Horses were fed 6 kg timothy hay, 3 kg unfortified sweet feed, 120g of a Ca-free vitamin-trace mineral supplement and 30 g loose salt per day. Horses were also given either 60 g/d BMC or 50 g/d CC so the total diet supplied ~ 45g Ca. Horses on OM were given a full tube of GastroGard® once daily for the final 14 d of each 21-d period, which supplied 3.91±0.17 mg/kg BW/d of OM. Feed and feces were analyzed for macronutrients, macrominerals and microminerals. Serum Ca, P, alkaline phosphatase, gastrin, PTH, and vitamin D were measured. Gastric fluid pH and ulcer score was measured on day 21.

Omeprazole administration had a profound effect on gastric fluid pH compared to nontreated horses (pH 6.0 ± 0.3 vs 2.0 ± 0.2) ($p<.001$), but

there was no effect of Ca source on gastric pH. Neither OM nor calcium source affected ulcer score. Serum gastrin levels were doubled in OM-treated horses compared to nontreated horses (53.8 ng/L ± 5.3 vs 26.7 ± 2.7 ng/L) ($p<.05$). There was a trend ($p=0.12$) towards higher PTH in OM-treated horses. Serum Ca, P, ionized Ca, vitamin D, and alkaline phosphatase were unaffected by OM or Ca source. OM and Ca source did not affect the digestibility of DM, CP, fat, ADF, NDF, starch, or WSC. OM and Ca source did not affect the digestibility of minerals except Ca. Ca digestibility was affected by both OM and Ca source. OM reduced apparent Ca digestibility from 52.0% to 41.4% in CC and from 55.1% to 46.5% in BMC. This is a 20% and 15% decrease in Ca digestibility in the CC and BMC diets. Mineral source had a significant effect on Ca digestibility with 50.8% for BMC and 46.7% for CC ($P<0.05$).
OM reduced Ca digestibility, resulting in an increase in dietary Ca requirements. Long-term omeprazole administration combined with other factors such as high-oxalate pastures or the regular use of furosemide may compromise calcium balance. Calcium balance is of special concern due to its vital role in skeletal health. Horses receiving OM should have Ca intake reviewed and may need extra Ca. The marine-derived Ca source BMC had higher digestibility in horses than CC and was less affected by OM, making it a more effective Ca source in horses on OM.

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A Marine-Derived Calcium Supplement Increases Bone Density in Thoroughbred Racehorses

P.J. Huntington, J.D. Pagan, A. Swanhall, E. Ford, and E. Mulvey

Bone density and strength are important for the long-term health and soundness of performance horses. Marine-derived calcium mineral complexes have been shown to improve bone density and strength in laboratory animals and humans. This study evaluated the effect of a supplement containing marine-derived calcium (Triacton™) (TA) on bone density in Thoroughbred racehorses. Thirteen horses (average age = 3.1 ± .4 y) were used in a 12-week training study. Horses were fed a timothy hay and fortified concentrate ration that supplied 64 g Ca and 42 g P/d. Seven horses received 120 g/d of TA which supplied an additional 15 g Ca and 7 horses received 120 g/day of a placebo pellet (CON). Training consisted of jogging, galloping, and breezing. There was no difference in training intensity or duration between the two treatment groups. Radiographs of the left front cannon bone were taken from a dorsal-palmar and a lateral-medial view at 0, 4, 8, and 12 weeks of the study. An aluminum step-wedge was placed in plane with the cannon bone to use as an external measure of bone density. Radiographic photodensitometry was used to measure the density of the lateral, medial, dorsal, and palmar cortices of the cannon bone. Differences due to training duration and supplementation were determined using a 2-way ANOVA. Over the 12-week training period, the horses supplemented with TA increased dorsal and palmar cortical bone density ($p<.05$). CON densities were not affected by training. Medial and lateral cortical bone densities were not affected by treatment.

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