HEALTHZONE

Nutrition



IF YOU ARE WHAT YOU EAT and hay is for horses, then what type of hay your horse eats must be important. So, too, of course is the grass in the pasture a horse grazes on and the type of feed it is given. All clichés aside, a horse's diet is an integral part of its overall health.

It is widely recommended a horse should eat on average at least 2% of its body weight in forage per day to maintain proper gut health. Horses are both herbivores and foragers, meaning they have evolved in such a way that allows them to utilize grasses and forage plants as a primary source of nutrition.

In general, hay falls into two categories, grasses and legumes, and horse hay is usually a mixture of both. The specific type of hay that an owner feeds typically depends on what is available in the region as well as the horse's lifestyle.

According to the American Association of Equine Practitioners, hay's nutritive value and palatability depend on a number of factors, including plant species; level of plant maturity at harvest; weed content; growing conditions such as rain, weather, insects, and disease; curing and harvesting conditions; soil conditions and fertility; moisture content; and length and method of storage.

Legume hays, such as alfalfa and clo-

ver, are often higher in protein, energy, calcium, and vitamin A than grass hays. This makes them best suited for younger horses who are still growing, lactating mares, and perfor-

mance horses.

Meanwhile, grass hays, such as timothy, are good for a wide variety of adult horses because they provide required roughage without the excess calories and protein.

Most people select hay by how it looks, smells, and feels, but it is important to consult a veterinarian or equine

nutritionist when adjusting or changing a horse's diet. Additionally, it is possible to have a certified forage laboratory analyze hay to determine its actual nutrient content, so when buying in bulk, that is something worth considering.

GRASS IS GREENER

In addition to consuming hay, grazing is often an important source of nutrition for horses. Unsurprisingly, one of the most coveted types of grass is the one that Kentucky is famous for—bluegrass but all types come with pros and cons, and it is harder to replace a bad pasture than a bad hay supplier.

"The type of grass that your horse is eating certainly does matter," said Dr. Jimmy Henning, a professor and forage specialist at the University of Kentucky. "In this part of the world, orchardgrass and bluegrass are what people desire to have, and they are good for horses for a multitude of reasons. Cool season grasses, such as orchard grass, bluegrass, and tall fescue, are going to be high in protein and energy. Tall fescue has plusses, but some significant minuses."

Grasses are typically divided into two categories. Cool season grasses grow from March until June and then September to November, while warm season grasses, such as Bermuda grass, grow

from June to October.

"The good thing about cool season grasses is that their calcium to phosphorus ratios tend to be 1:1, which is not a bad ratio to feed," said Henning. "You typically need 2:1 if you are talking about a whole diet for maximum bone strength, so 1:1 for the grass is not bad since a horse probably isn't getting all its

nutrition from the pasture.

"The minerals are going to be what they are, but minerals in forages are not readily all available. They will supply some, but this is a clear case where you *(continued on page 54)*

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University of Kentucky's Dr. Jimmy Henning

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MicroRNAs as Novel Biomarkers or Insulin Resistance



DR. TANJA HESS & DR. REBECCA SPLAN

ONE SENTENCE SUMMARY: In an effort to improve the diagnosis, management, and treatment of the many horses and ponies who suffer from metabolic disorders related to insulin dysregulation, we will investigate the ability of novel, small, generegulating molecules called microRNAs to be used as convenient and accurate biomarkers for equine insulin resistance.

Insulin resistance occurs when circulating concentrations of the hormone insulin fail to exert their normal biologic response, setting the stage for serious metabolic complications, acute or chronic illness, and even death. Normally, in response to a meal or feeding challenge, the combined effect of increased blood glucose concentrations and action from hormones will stimulate insulin secretion from the pancreas. In a healthy horse this insulin enters the bloodstream and initiates a cascade of events at the cellular level to shuttle circulating glucose into tissues; primarily in skeletal muscle and the liver, where it is stored as glycogen, and in adipose tissue where it is stored as fat. As a result, circulating levels of glucose are kept within a narrow window compatible with normal metabolic functioning.

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However, in some animals, the system breaks down, causing IR. Exact mechanisms for how, why, and when IR occurs remain unknown. A multitude of factors might play a part. Disturbances in insulin effectiveness might affect systems that regulate immunology, circulation, reproduction, growth, and more. For this reason, IR in horses is associated with a number of debilitating disorders, including equine metabolic syndrome, pituitary pars, intermedia dysfunction, and laminitis.

Our current diagnosis of IR is done most accurately by performing tests such as the euglycemic hyperinsulinemic clamp or the frequently sampled intra-venous glucose tolerance test with the minimal model analysis. However, these tests are expensive and labor intensive, requiring large numbers of samples, significant time commitment, and complicated analytical modeling.

Since their discovery in 1993, micro-RNAs (miRNAs) have emerged as exciting key regulators of biological processes and promising biomarkers for disease. These small (~22 nucleotides in length) RNA molecules bind to specific mRNA targets and either promote their degradation or stop their ability to code for proteins. In most species there are few miRNAs relative to the number of mRNAs they might regulate. For example, in the horse there might be more than 25,000 genes and associated mRNAs, but the number of miRNAs is predicted to be ~1000. This is because one miRNA might regulate hundreds of mRNAs, and thus single miRNAs might have a substantial influence. For this reason, differential miRNA expression patterns between healthy and diseased animals are especially rich in information.

MiRNA profiling has become a powerful tool as miRNAs have been shown to be incredibly robust in circulation and are measurable to a much higher sensitivity than cell-free circulating proteins. For this reason, miRNA profiling is of great interest to scientists wishing to develop biomarkers for diverse biomedical applications, including cancer, autoimmune, cardiovascular, and metabolic diseases. In human metabolic research, for example, miRNAs have already been identified that can separate groups of patients into clinicallyrelevant classes, such as pre-diabetic or type 1 diabetic cohorts, and a large suite of miRNAs has been implicated in aberrant beta-cell development and functioning.

In this study we apply this novel tech-

nology to insulin resistance, a complex and causative factor in the development of several debilitating medical conditions with large economic impact to the horse industry. Identification of miRNAs responsible for "fine tuning" expression of vast numbers of target genes is a cost-effective method that will not only provide new and useful information on pathogenesis of equine disease but also give insight into biomarkers associated with IR processes and progression.

We accomplished this by investigating the circulating miRNA profile of insulin resistant horses. We hypothesized that insulin resistant horses would have a different circulating miRNA profile than those that were healthy. Twelve Thoroughbred/ Thoroughbred-cross and 12 Welsh/Dartmoor non-pregnant mares were evaluated for insulin sensitivity using frequent sampling intravenous glucose tolerance tests. Serum samples were collected for miRNA profiling. Mares were divided into healthy or IR groups and their miRNA profiles compared. MiRNA profiles of horses and ponies were also compared. Results demonstrated that 13 miRNAs were differently expressed between IR and IS horses, 15 between IR and IS ponies, 17 between horses and ponies, and 10 between IR and IS animals (horses and ponies combined) (p<0.05). Finally, 8 miRNAs were proposed as potential regulators of equine insulin resistance. In addition to our preliminary investigation, this study suggests potential miRNA profiling is a potential new tool to be used to further understand the mechanisms involved in equine insulin resistance and associated conditions. BH

Dr. Tanja Hess is an associate professor at Colorado State University and Dr. Rebecca Splan is an associate professor at Virginia Tech.



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HEALTHZONE Nutrition

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are probably feeding some sort of salt block, mineral block, or something in the barn to make sure they are getting the right ratio of calcium to phosphorus."

As the name suggests, one of the problems with cool season grasses is that they can struggle in the height of summer. That tendency is one of the knocks against bluegrass, although orchard grass tends to be a bit hardier.

"The thing you would change about bluegrass if you could is the fact that it is dormant in the summer," said Henning. "It really doesn't like hot temperatures. Orchard grass to some degree doesn't like it either, but it will do a lot better in July and August than bluegrass. The bluegrass will just turn brown, while the orchard grass will at least stay green except for exceptional droughts."

As one might expect, geographic location is going to be the biggest factor in what grasses grow well in any given region. In addition to Bermuda grass, according to the University of Florida, some quality warm season grasses to consider are Bahia grass, which can withstand heavy grazing, and pearl millet, which is higher in nutritional value than most perennial grasses. Because of the weather there, grazing is a viable option almost all year-round.

"Florida's unique climatic characteristics allow for forage production most of the year with a large variety of possible forage choices," explained Y.C. Newman, et al., in the research paper "Pastures and Forage Crops for Horses." "Independent of the size of the operation and number of horses, good forage planning can help reduce feeding costs, environmental impacts, and nutritional disorders caused by high-concentrate feeding. With the exception of high-performance animals, horses can meet most of their nutritional needs from pasture."

DOUBLE DUTY

The nutritional value of the grass is not the only thing farm managers need to consider when evaluating grass types. Because horses will be turned out on the fields constantly, how the grass grows is also a key practical factor.

"If it grows once and it doesn't grow back, that's not a good pasture grass even if it was nutritious," said Henning. "Pasture grass has got to do a lot more than just feed the animal, at least for horses. Being able to grow across a wide range of conditions-including soil, weather, growing season-all of those things come into play. Orchard grass and bluegrass are very good at that, lending to their popularity."

No grass is perfect though, and while orchard grass handles





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heat better than bluegrass, it does not spread very much and its life cycle is more limited than desired. Often it is mixed with bluegrass to balance these weaknesses.

"Bluegrass will withstand a lot of traffic," said Henning. "You can even work it up to a mud lot, and it will try to come back at least once. It is a natural spreading grass. It's a true sod forming grass, which makes it good for this, and it gives good hoof support."

Orchard grass, on the other hand, grows in clumps and does not spread. It grows well where it grows, but that's it.

"It is an erect bunch grass, which means that's just the way it's going to look," said Henning. "In a mature bluegrass/orchard grass pasture, you are going to have clumps of orchard grass and in between you are going to have bluegrass and/or clover and/or weeds. That's the way it is going to play out. Orchard grass doesn't spread at all. The other downside is it doesn't last as long as you would like it to. People want it to last forever, but chances are four to six years for an individual plant is all you are going to get out of orchard grass.

"Bluegrass and orchard grass are still the prime candidates though. They provide good nutrition, they are desired by the horse, and they don't have any toxicity issues."

DANGER ZONE

Toxicity issues restrict the popularity of another well growing grass, but research is working to change that.

One of the best growing grasses in America is tall fescue—it is growing on approximately 35 million acres of land but it has proved to be dangerous for some horses. The grass itself is fine, but it is usually infected with a toxic endophyte, and a study done by Clemson University showed that fescue toxins undoubtably have an effect on pregnant mares.

Removing a broodmare from tall fescue three months before her foaling date has proved to be an effective method of maintaining her health. If she is not taken off tall fescue, significant problems that have





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been traced to the grass can occur, including lower conception rates, abortions, prolonged pregnancy, foaling difficulties such as dystocia, thick or retained placenta, poor milk production, higher rates of newborn foal death, and increased risk of laminitis.

"Fescue toxicity is the most common thing that can cause prolonged gestation and reduced milk production," explained the AAEP's Dr. Benjamin Espy. "But by the due date it is too late to restrict the fescue grazing of the mare since it usually requires 60 to 90 days of restricted grazing to make a difference."

The endophyte steadily poisons horses that ingest it. Horses are extremely sensitive to the toxin, which is called ergovaline, and according to the AAEP, fields with as little as 5-10% infection rates can pose potential hazards to broodmares and foals.



Tall fescue lasts longer than orchard grass

"It is one of the biological mysteries for us—why or how did the original field of Kentucky 31 tall fescue get infected with this toxic endophyte?" said Henning. "The tall fescue that just occurs naturally, if it's just there, chances are it is the toxic type. We know that is certainly incredibly bad pasture for mares in late gestation."

Tall fescue in and of itself does have some plusses to it, however, one of which is that it stands up well to constant use.

"Like bluegrass, tall fescue is more traffic tolerant than orchard grass," said Henning. "Blades of orchard grass are going to decompose during the winter. It's not going to stay intact. A tall fescue leaf may turn yellow or bleach out to white, but it is going to stay intact. It may be matted in the pasture, but it is still going to be there, which you may need for hoof support."

Because of its good qualities, tall fescue has not been completely written off, especially if farms are confident that their particular fields are not infected with the toxic endophytes.

"There are naturally occurring non-toxic endophytes, and those are the ones being sold as novel or friendly endophyte tall fescues," said Henning. "Farms that have gone to using novel endophyte tall fescue have done so because they have worked with a pasture program to kill a field completely down to bare ground. They know they have killed the old tall fescue.

"They want the novel endophyte tall fescue because it is

tough. It lasts longer than orchard grass, so you aren't having to worry about when your orchard grass is going to die. That's why they are converting to it, slowly."

KNOW WHAT IS GROWING

As they have with hay, managers interested in knowing the scientific specifics of what is growing in their fields can find out. For example, in 2005 the University of Kentucky introduced the Horse Pasture Evaluation Program. The program has performed more than 200 evaluations, representing more than 40,000 farm acres in 21 counties across the commonwealth.

The team goes to a farm and examines its fields in order to provide detailed pasture management recommendations. The goals are to improve pasture land by increasing forage quality and quantity; reduce the need for stored feeds such as hay and grain; and assess the potential risk of fescue toxicity for pregnant broodmares.

"It is an area where farm managers are not as informed as they want to be," said Henning. "There is a real need for farm managers to know exactly what grasses are in their pastures, and that is one of the reasons the program is so useful.

"One of the major value points for that program is it will tell you of all the plants out there, what percentage is fescue in a given pasture and where it is. They will also measure the presence or absence of the endophyte, and they will measure at a given point of time the ergovaline content. We will know how toxic that pasture is in the month it is tested. There is a cycle, so it varies month to month."

Knowing which fields are safe and which are not is an important management tool. "Twenty years ago, and I've been doing this longer than 20 years, you would be on a farm, look down, see tall fescue, and know it was not a good thing, but we didn't have a very nuanced approach," said Henning. "Now there is a much better understanding. You can say, 'Hey, look, do not let them graze this area within this window of time, and here is how we can make it better.'

"It's impossible to say how valuable the information on tall fescue content, infection, and ergovaline numbers is because the value of some of the animals we are talking about gets into the hundreds of thousands of dollars in a hurry. It is one of the few times in a university setting that advice can really be that impactful, that fast."

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