

The Comparative Intake and Nutrient Digestibility of Legume/Grass Hays in Horses

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Introduction:

The equine industry in the United States is an industry of rapid growth. The horse population as reported farms was 3,020,117 in 1997 and was updated to 3,644,278 by 2002 (USDA, 2005). Montana reported a growth from 56,364 horses in 1992 to 80,947 horses in 1997 to 94,396 horses in 2002 (USDA, 2005). This is creating a large industry in the United States for specialty items produced for equines. Among these specialty items is the very lucrative business of specialty hay farming. Currently specialty hays such as grass/legume hay mixtures are a high-value product. Most of the hay in the United States is sold in 51 lb bales (USDA, 2001). In 2001, Montana reported a value of \$85.00 to \$90.00 per ton for large bales and \$105.00 to \$120.00 per ton for smaller bales (USDA, 2001).

Horses weigh on average 500 kg or 1100 lb and consume 2% of their body weight in forage (NRC, 1996). With vast numbers of horses consuming 2% of their body weight in forage, a large industry for hay producers is being created. The objectives of this study are to 1) determine digestibility of legume/grass hay blends, 2) determine if a preference exists between the hay blends, and 3) determine if a correlation exists between digestibility and preference. The hypothesis of this study is that horses have a preference for certain hays and this should correlate with digestibility of the preferred hay blend

Literature Review:

The two main groups of ungulates today, consist of ruminants and non-ruminants. These two groups have similarities and differences in their digestive processes. Both have similar fermentation processes and similar amounts of volatile fatty acids (VFA's) being produced (Janis, 1976). The VFA's produced from fermentation in the horse are absorbed in the cecum and large colon, whereas the VFA's produced from similar processes in the cow are absorbed through the rumen wall as soon as they are formed (Janis, 1976).

Ruminants and non-ruminants differ in the sites of protein and carbohydrate metabolism as well as the length of time feed is in the fermentation site. By the time the feed reaches the cecum in the horse, a large amount of the available proteins and carbohydrates have been absorbed in the small intestine (Lewis 1995, Janis 1976). In the cow, carbohydrates and proteins cannot avoid being fermented along with cellulose before the animal has a chance to absorb them.

Fermentation in the horse, takes place in the enlarged cecum and large colon (Lewis, 1995, Janis 1976). The cecum itself holds 7-8 gallons of feed material and the large colon can hold 14-16 gallons of feed (Lewis, 1995). It is here that the bacteria break down the cellulose in forages fed to horses. Lignin makes up 12-18% of the polysaccharides from this breakdown unavailable to the horse (Lewis 1995, Janis, 1976). Horses will naturally select a high fiber and low protein feed and cecal digestion is an adaptation for the high fiber content feed consumed by the horse (Lewis 1995, Janis 1976).

Dietary Energy (DE) is very important in the horse and they derive the majority (50-80%) of their DE from carbohydrates in forages and grains (Longland, 1997). Plant carbohydrates are made up of four components that consist of 1) free sugars such as glucose, sucrose and fructose, 2) oligosaccharides, 3) storage polysaccharides such as starch and fructans, and 4) cell wall carbohydrates such as non-starch polysaccharides (Longland, 1997). The horse uses this dietary energy to produce heat and ATP (adenosine triphosphate), which cells use to function (Longland, 1997). The starch component of the feed is hydrolyzed to glucose by the horse's enzymes in the small intestine, where when combined with free sugars, can be absorbed and metabolized to yield ATP (Longland, 1997). Therefore the energy value of a feed will depend on the mounts of digestible and fermentable substrates that it contains (Longland, 1997).

Digestibility of feeds can be determined by using an analysis for marker (Van Keulen et al., 1977). The two types of markers are internal and external markers. Internal markers are indigestible substances that are present in the feed e.g., fecal nitrogen index, lignin, chromogen, acid insoluble ash (AIA), and indigestible fiber (Barbisan, 1993). External markers are indigestible components that are added to the feed or administered orally, e.g., chromic oxide, ferric oxide, silver sulfide, polyethylene glycol, and preparations of chromium, cobalt, hafnium, and several of the rare earth metals (Barbisan, 1993). External markers require total fecal collections, whereas internal markers only require grab samples (Van Keulen et al., 1977). Barbisan (1993) reported that using AIA in horses was as reliable as external markers and more convenient. McCarthy et al., (1974) evaluated AIA to be superior to chromic oxide used in swine.

It has been reported that horses do in fact have a preference among plant species throughout the year in a grazing community (Nash, 1997). Another preference study for alfalfa-grass hay found significant results ($p = .001$) in preference between am and pm cuttings (MacKay et al., 2003). Preference of horses for different typed of hays can be measured by intake and refusals (MacKay et al., 2003). Potential voluntary intake can be influenced by several factors. Factors that influence intake include palatability, digestibility, rate of passage, chemical composition, and physical factors characteristic of the forage (LaCasha et al., 1999). Intake of legumes is generally higher than that of grass hays, even at the same digestibility. This is due to the fact that legumes usually have a lower cell wall concentration and a higher rate of passage (LaCasha et al., 1999, Allison 1985). Intake is dependent on the structural volume which in turn, includes cell wall content (Allison, 1985). The cell wall of plants includes Neutral Detergent Fiber (NDF)

and Acid Detergent Fiber (ADF). The NDF portion contains acid detergent solubles such as hemicellulose and acid detergent fiber which consists of cellulose and lignin. Hemicellulose and cellulose both contain nutrients that are partially digestible. Lignin, however, is indigestible and nutrients from this are unavailable (Owensby, 1991). The higher the cell wall content is in ADF and NDF, the less digestible the plant becomes and slows the rate of passage, therefore increasing time needed to process the feed (Owensby, 1991). It has also been suggested that certain plant attributes, such as low protein content or nitrogen deficiency, result in a reduction of intake (Allison, 1985). Voluntary intake also correlates with digestibility. The rate of passage for ruminants has been shown to increase with increased digestibility (Allison, 1985). Forage intake is controlled by rate of passage up to about 66% digestibility, but above those levels, other factors are involved (Conrad, 1966). Other factors that can effect what an animal will choose include body size, age and physiological status. It has been shown that calves will choose a diet higher in crude protein and lower in ADF than older cows (Allison, 1985). Younger animals will consume higher quality forage due to their energy needs being larger than that of older animals (Allison, 1985). Allison (1985) has also reported that lactating ewes will require as much as 25-50% greater dry matter intake than a dry or pregnant ewe. Preferences exhibited by animals is only considered to be important when one feed is consumed in a larger quantity, exhibits a higher digestibility or both (Burns et al., 2005)

Preference trials consisting of more than two decisions per feeding period may affect the outcome due to the fact that horses will generally pick the feed closest to them (Fitzgerald 2004, Buntinx et al., 1997, Burns et al., 2005). Therefore, no more than two containers of feed should be offered to a horse per feeding period. It has been suggested that more preference studies be completed in equines (KER 2004, Kennedy et al., 2004).

Experimental Methods

Digestibility Trial:

The trial was conducted during a 36 d. period located at the Oscar Thomas Nutrition Center at Montana State University- Bozeman. Four horses of the same breed, sex, lineage, and with similar body weight and age were fed four different grass/legume hay blends. The hay blends fed included orchardgrass (*Dactylis glomerata* L.)/alfalfa (*Medicago sativa* L.) (OA), orchardgrass/sainfoin (*Onobrychis viciifolia* Scop.) (OS), timothy (*Phleum pratense* L.)/alfalfa (TA), and timothy/sainfoin (TS). Each hay blend was grown from 1/2-acre plots located at the Northwestern Agricultural Research Center located in Creston, Montana. The first cutting hay blends were harvested in June of 2003 and 2004. The second cutting hay blends were harvested in August of 2003 and 2004. The feeds were shipped to Bozeman, Montana where they were fed to four horses. These four hay blends were sampled with a core forage sampler and tested for CP, ADF, NDF, DM, and OM.

The feeding trial is a 4 x 4 Latin square design, with horses placed in individual pens with access to ad. Libitum water and mineral. The horses had a 4-d work up period and a 5-d collection period for each hay blend. The 4 -d work up period allowed each

horse to pass any previous digestive material before the collection began. The 5-d collection period followed, during which representative hay samples, total refusals, and fecal excretions were collected. Grab samples were taken from the hay as fed to each animal at 0800 h and 1600 h. Fecal grab samples were collected twice daily at random times during the day. Collected samples were dried immediately at 50°C for 48 hours. The dried feces and hay samples were ground to pass a 1-mm screen (Wiley Mill, Thomas Scientific, Boston, MA).

Feed, refusals, and fecal samples were analyzed to determine percentage CP, NDF, ADF, DM, OM, and acid insoluble ash (Goering and Van Soest, 1970). Total ash was estimated by ashing samples in a muffle oven at 580°C for 10 hours.

Preference Trial:

The preference trial was completed at the Oscar Thomas Nutrition Center located in Bozeman, Montana. The study consisted of six horses of the same breed, sex, lineage, and with similar body weight and age range. All four hay blends were sampled with a core forage sampler and tested for CP, ADF, NDF, DM, and OM. Horses were placed in individual pens and had access to ad. Libitum water and mineral. Each horse was offered two different hay combinations per feeding period and had all six combinations of feed when the trial was completed. The six hay combinations included OS/TA, OA/TA, TA/TS, OS/TS, OA/TS, and OA/OS. Horses were tied up and feed was placed in two identical feed tubs located at the front of each pen. Once all tubs were filled, the horses were released and allowed to choose from the two hay blends. Each animal was given 1.5 hours to consume a half ration of feed.

Horses were fed during a 3-d period and the study was replicated once more with a different set of six horses for a total of 6 days. The first trial was completed using geldings whereas the second trial was completed using mares. During each 3-d period, intake was measured by weighing the amount of feed fed as well as total refusals.

Statistical analysis:

All data from the digestibility trial was analyzed as a 4 x 4 Latin square design. The model included terms for animal, period and treatment. Time was later removed from the model as the experiment was found to be confounded by time. However, environmental factors did not differ during the time that the trial took place, so time should not have been a significant factor. The data from the digestibility trial were analyzed using the GLM procedure in SAS. The model included terms for animal and treatment. The two-way interaction was used to test all sources of variation for significance. Means for all variables analyzed were compared using Duncan's Multiple Range Test in SAS. All forage and fecal composition data were considered significant at $P \leq 0.05$.

The data from the preference trial was analyzed as a 6 x 6 Latin square design. The model included terms for animal, period and treatment. The data from the preference trial was analyzed using Latin square GLM procedure, simple ANOV mixed procedure, and MDS (multidimensional scaling) analysis all in SAS. These procedures were used to

test all sources of variation for significance. The Latin square GLM procedure used Waller-Duncan Grouping to analyze means. The simple ANOV mixed procedure used contrasts to compare the preference of hay types. MDS analysis uses intake data obtained from the preference trial and computes ratios using the following calculation: $\Delta = (\text{Intake of preferred forage} - \text{Intake of less-preferred forage}) / \text{Total intake}$. Data was considered significant at $P \leq 0.05$. The delta value for each pair is expressed as a difference ratio from 0 to 1 (Buntinx et al., 1997). This model then arranges forages by estimating coordinates in orthogonal dimensions in order to model the differences (Buntinx et al., 1997). Hays with coordinates that are found to be similar in preference and also coordinates that are far apart in the dimensional space indicate that there is a difference in preference.

Results and Discussion

Three separate experiments were conducted, including the digestibility trial, the preference trial for geldings and the preference trial for mares.

Digestibility Trial

The model for the digestibility trial using the GLM procedure proved to be significant for both hay and horse with $P \leq 0.0006$. The dependent variable of AIA feed was significant for hay type with $P \leq 0.0001$, meaning that there were significant differences between hay types for digestibility. This model proved that horse was an insignificant class for the dependent variable of hay AIA. The dependent variable of fecal AIA was also significant for hay type with $P \leq 0.0012$, meaning that there were significant differences between hay types for the digestibility demonstrated by fecal AIA. The model showed that horse was an insignificant class for the dependent variable of fecal AIA. The Duncan grouping compared the means and found that orchardgrass/sainfoin was significantly different in digestibility from the other three hays. There were no differences in digestibility between orchardgrass/alfalfa and timothy/sainfoin. There were also no differences in digestibility between timothy/sainfoin and timothy/alfalfa. The Duncan grouping also compared the means of the fecal AIA and found that timothy/alfalfa was significantly different in digestibility from the other three hays. There were no differences in digestibility between timothy/sainfoin and orchardgrass/alfalfa. There were also no differences in digestibility between orchardgrass/alfalfa and orchardgrass/sainfoin for fecal AIA.

When compared to the nutrient content analysis, orchardgrass/sainfoin had an average dry matter NDF content of 50.35 g and a dry matter ADF of 32.86 g. These were the highest values of all four hays and since both digestibility and intake decrease with an increase in ADF and NDF it would explain why orchardgrass/sainfoin was found to be significantly different in digestibility of the feed. Timothy/alfalfa was found to have the lowest amounts of NDF and ADF with dry matter values of 40.38 g and 28.26 g respectively. This would explain why timothy/alfalfa was significantly different from the other three hays in fecal AIA. Timothy/alfalfa should be the most digestible feed out of all hay combinations and this was supported by both the nutrient analysis and the SAS analysis.

Preference Trial Geldings

The Latin square GLM procedure analysis showed that there were no differences in the mean quantity of all hays consumed as a total. All hay combinations were consumed in similar quantities, but the horses did differentiate between the six hay combinations.

The simple ANOV mixed procedure analysis found that when contrasted, there was a significant difference between alfalfa hays and sainfoin hays, with $P \leq 0.0004$. There were no significant differences detected between orchardgrass hays and timothy hays. There were also no significant differences between legume hays and grass hays. The geldings in this preference trial sought out the hays that contained alfalfa and found those to be the preferred hay types.

The MDS analysis for the gelding preference trial fits the data from the ANOV into a mapping of distance correlations. Based on this figure, the geldings showed a preference for both timothy/alfalfa and orchardgrass/alfalfa, but based on MDS, for different reasons. The x-axis appears to be the "timothy axis" and the y-axis appears to be the "orchardgrass axis" in the case of the geldings. Anything in the upper right hand corner of the graph is indicated to be positive, whereas anything in the lower left hand corner is assumed to be a negative preference. Although animal-to-animal variation was present, the preference here was expressed for alfalfa first and orchard grass second. Timothy/sainfoin was found to be the least preferred hay.

The nutrient analysis of the hays reported that orchardgrass/alfalfa and timothy/alfalfa were the least digestible feeds with average NDF values of 45.95 g and 49.19 g respectively and average ADF values of 36.76 g and 33.43g respectively. The more digestible hays were found to be orchardgrass/sainfoin and timothy/sainfoin. The geldings in this trial most probably chose the least digestible alfalfa blends because alfalfa alone was the most digestible. However, when added to the grass, digestibility may have decreased. This has not been proven due to the fact that the hays were tested as a mixture instead of being tested by themselves.

Preference Trial Mares

The Latin square GLM procedure analysis for the mare preference trial showed very little differences in the mean quantity of hays consumed as a total. Using the Waller-Duncun grouping, it was found that all hays were consumed in similar quantities, but the horses did differentiate between the six hay combinations.

The simple ANOV mixed procedure analysis found that when contrasted there was a significant difference between orchardgrass hays and timothy hays, with $P \leq 0.0239$. There were no significant differences found between alfalfa and sainfoin hays and also no significant difference was detected between legume and grass hays. The mares sought out hays that contained orchardgrass and found those hays to be the most preferred hay types.

The MDS analysis for the mare preference trial fits the data from the ANOV into a mapping of distance correlations. Based on this figure, the mares displayed a preference for orchardgrass with less concern for alfalfa. Timothy/sainfoin was also found to be the least preferred hay. The preference for hays by the mares was orchardgrass blends first and alfalfa blends second.

The nutrient analysis of the hays used in the mare preference trial reported that orchardgrass/sainfoin and orchardgrass/alfalfa were the least digestible hays with average NDF values of 46.71 g and 51.66 g respectively and average ADF values of 34.03 g and 39.48 g respectively. Timothy/alfalfa and timothy/sainfoin both demonstrated lower ADF and NDF values and were found to be the more digestible hay types. Again, the explanation for the mares choosing the two least digestible feeds is probably due to the orchardgrass being more digestible, but when added to the legumes, digestibility decreases. Hays would need to be analyzed as singles instead of blends in order for this statement to hold true.

The hypothesis that horses have a preference for certain hays would be accepted as shown above by the preference studies. The hypothesis that the preference for hays correlates with the digestibility of hays would be rejected, as both the geldings and mares chose hay mixtures that were least digestible.

Implications

According to the digestibility trial, nutrient testing of hays proves to correlate with actual digestibility in the horse. The ADF, NDF, CP, OM and DM analysis of the hays proved to be a good indicator of digestibility. More research is need in the area of preference for this experiment. Each individual hay needs to be analyzed by itself instead of as a mixture. This would be a better indicator of why the horses chose the feed types that they did. The trial did demonstrate that horses do have a preference for certain hay types. There is a large industry being created for specialty hay products. Hay producers could capitalize on this industry once a particular hay type has been found to be the most preferred.

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