Final Report

Title: Legume-Grass Mixtures for Improved Horse Pastures

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Abstract: (Limit 200-300 words)
Cool-season perennial grasses are the foundation of productive horse pastures in the North Central U.S.; however, many strive to add legumes to increase yield and digestible energy. The objective of the research was to evaluate forage nutritive value, yield, and preference of pure grass and mixed grass legume pastures under horse grazing. Plots consisting of orchardgrass, Kentucky bluegrass, meadow fescue, and binary mixtures of the three grasses with alfalfa, white clover and birdsfoot trefoil were grazed by adult horses in the summer 2013. Yield, forage nutritive value and horse preference were determined and corrected for percent weeds. Heavy infestation by weeds was observed in most plots and ranged from 9 to 95%. The white clover meadow fescue mixture, alfalfa orchardgrass mixture, and birdsfoot trefoil meadow fescue mixture were most preferred with 76 to 88% removal. The alfalfa Kentucky bluegrass mixture, birdsfoot trefoil Kentucky bluegrass mixture, and Kentucky bluegrass were the least preferred forages with 29 to 38% removal. The birdsfoot trefoil and meadow fescue, and white clover and orchardgrass mixtures produced the most yield (≥ 1.39 tons/acre), while the birdsfoot trefoil and Kentucky bluegrass mixture, and Kentucky bluegrass produced the least amount of yield (≤ 0.19 tons/acre). Forage nutritive values were different among mixtures and species. The amount of CP ranged from 20 to 25%, ADF ranged from 24 to 34%, NDF ranged from 36 to 62%, NSC ranged from 21 to 7%, and Equine DE ranged from 1.2 to 0.93 Mcal/lb. The Ca:P ranged from 3:1 to 1:2. Although weed invasion made it difficult to interpret results, it appears that white clover mixed with Kentucky bluegrass, orchardgrass or meadow fescue may present a viable option for horse owners wanting to plant a cool season grass legume mixed pasture.

Introduction
Cool-season perennial grasses are the foundation of productive horse pastures in the North Central U.S. Many livestock owners strive to add legumes to grass pastures to increase yield and forage nutritive components like crude protein and digestible energy. However, legume-grass mixtures are rarely evaluated under livetock grazing for nutritive value, yield, and animal preference. Horses are known to be highly selective and aggressive grazers, especially compared to other livestock (Archer, 1973). Differences in preference, defined as the behavioral response of an animal to plants when a choice is given (Marten et al., 1987), affects animal utilization of a species as well as forage yield.

Along with preference, forage nutritive value is an important factor affecting pasture utilization. It is commonly known that grass and legume species have different chemical compositions. Legumes tend to have a lower nonstructural carbohydrate component and higher crude protein,

calcium, and digestible energy contents compared to cool-season grasses when grazed or harvested at a similar maturity. Furthermore, newer, more productive species and varieties are available for pasture use but have not been extensively evaluated in mixtures or under livestock grazing. The objective of this research is to evaluate forage nutritive value, yield, and preference of pure grass and mixed grass-legume pastures under horse grazing.

Materials and Methods

In August 2012, four replicated small pastures (11 x 26.8 m) consisting of twelve plots (3.7 x 6.7 m) each were seeded at the equine research pastures at the University of Minnesota in St. Paul, MN. The pastures consist of three single grass sward plots: orchardgrass, Kentucky bluegrass and meadow fescue. The remaining nine plots in each pasture consist of binary mixtures of the three grasses with three legumes: grazing tolerant alfalfa, grazing tolerant white clover and birdsfoot trefoil. In April 2013, winter injury of orchardgrass, meadow fescue and alfalfa was observed; all plots were over-seeded. All plots were fertilized according to soil test recommendations according to University of Minnesota recommendations.

Grazing was initiated on July 23 and August 13, 2013, when cool-season grasses reached 15 to 20 cm. Grazing did not occur beyond August 2013. Prior to grazing, forage yield was determined by mechanically harvesting a 0.9 m x 3.7 m strip from each plot (Figure 1). A sub-sample was dried, and yields were cumulated and reported on a dry matter basis and corrected for percent weeds. Forage nutritive value was determined on July 22, 2013, by hand-harvesting a sub-sample to a 9 cm height from one random 0.25 m^2 area (Figure 2). Due to heavy infestation by weed species, sub-samples were hand sorted into grasses, legumes and weeds (Figure 3). The grass and legume components of the separated sub-samples were dried and weighed. Grass and legume components were ground, combined (where appropriate), mixed well, and submitted for forage nutritive value analysis including crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF), nonstructural carbohydrates (NSC; estimated by adding starch and water soluble carbohydrates), calcium (Ca), phosphorous (P), and equine digestible energy (Equine DE) via NIRS. Weed components were discarded and not included in forage nutritive value or yield.

Figure 1. Harvesting plots for yield

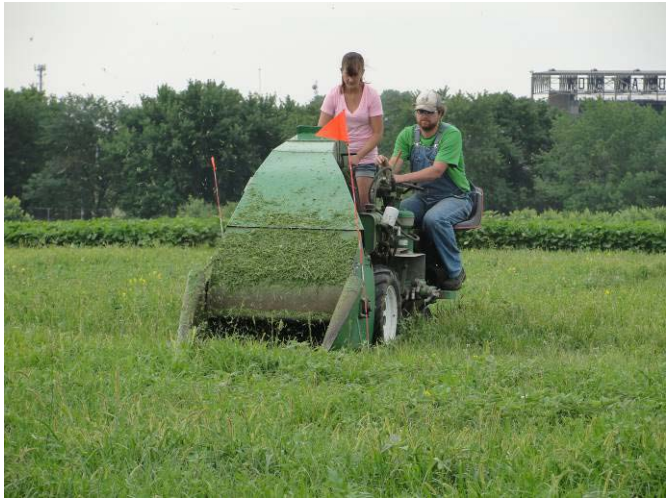
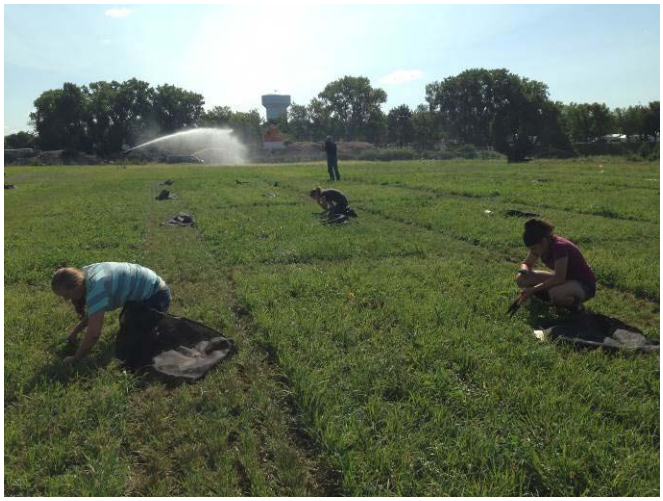


Figure 2. Hand-harvesting plots for quality



Horses were acclimated to grazing grass pastures for four hours a day for two weeks. Starting on July 23 and August 13, 2013, four adult stock-type horses grazed one replicate for four hours (0800 to 1200 hours), switching to the next replicate each subsequent day for four days, until the horses had grazed all four replicates (Figure 4). Upon rotation of horses off the pastures, pastures were visually assessed for removal (to determine preference) on a scale of 0 (no grazing activity) to 100 (100% of the plants were grazed down to 9 cm); visual removal of weeds was not taken into consideration (Allen et al., 2013). After each grazing event, manure was removed and pastures were mowed to 9 cm and allowed to re-grow. When not grazing, horses were housed in a drylot and fed grass-alfalfa mixed hay twice daily at 2.0% of the herd body weight (NRC, 2007). Ad libitum water and access to a trace mineralized salt block were provided in the drylot and while on pasture.

Figure 3. Hand-sorting samples

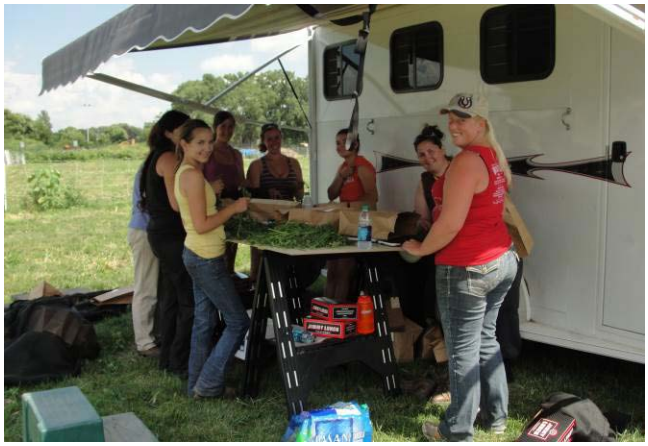
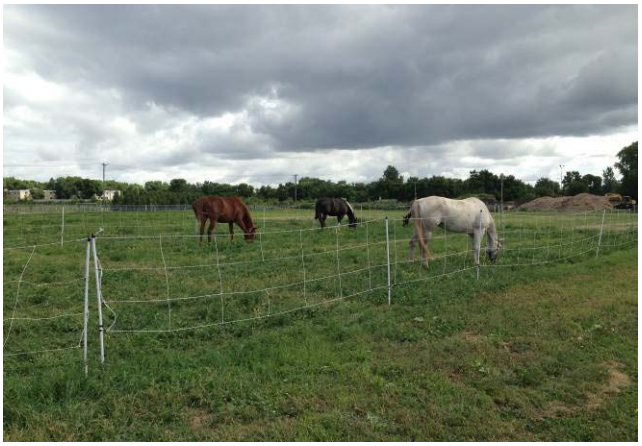


Figure 4. Horses grazing research plots



Data was analyzed with Statistix with plot as the experimental unit. Statistical significance was set at $P \leq 0.05$.

Results and Discussion

Heavy infestation by weeds was observed in most plots and ranged from 9 to 95% (Table 1), despite pre- and post-planting measures to control weeds. These measures included a pre-plant application of glyphosate, multiple tillage passes, over-seeding, and multiple post-emergence mowing events prior to grazing. One major concern with planting mixed legume grass pastures is weed control. No herbicide exists that will selectively control weed species while not injuring the desired grass and legume species. Livestock owners should be aware of weed control issues when planting a mixed legume grass pasture.

During the summer of 2013, horses showed distinct preferences among the grasses and mixtures ($P \leq 0.05$; Table 2). The white clover meadow fescue mixture, alfalfa orchardgrass mixture, and birdsfoot trefoil meadow fescue mixture (Figure 5 and 6) were most preferred by the horses with 76 to 88% visual removal. The alfalfa Kentucky bluegrass mixture, birdsfoot trefoil Kentucky bluegrasss mixture, and Kentucky bluegrass planted as a monoculture were the least preferred forages by the horses with 29 to 38% visual removal. Other mixtures and monocultures were

moderately preferred with visual removals of 64 to 54%. Generally speaking, legumes mixed with meadow fescue tended to be highly preferred, followed by orchardgrass then Kentucky bluegrass. However, weed infestation likely impacted results as all plots containing Kentucky bluegrass had greater than 50% weed infestation. Therefore, the authors caution use of results to make broad recommendations on horse preference of grass legume mixtures.

Table 1. Percent grass, legume and weeds in pastures on July 22, 2013.

	Grass	Legume	Weeds
	% of stand		
Alfalfa Kentucky bluegrass mixture	5	19	76
Alfalfa meadow fescue mixture	55	10	35
Alfalfa orchardgrass mixture	66	8	26
Birdsfoot trefoil Kentucky bluegrass mixture	4	8	88
Birdsfoot trefoil meadow fescue mixture	69	6	25
Birdsfoot trefoil orchardgrass mixture	62	4	34
White clover Kentucky bluegrass mixture	16	32	52
White clover meadow fescue mixture	47	20	33
White clover orchardgrass mixture	78	13	9
Kentucky bluegrass	5	-	95
Meadow fescue	60	-	40
Orchardgrass	64	-	36

Similar to the results in the current study, Allen et al. (2013) also observed horses preferred meadow fescue when given a choice among 12 different cool-season grasses. Casler and Van Santen (2001) also found that cattle preferred meadow fescue over other cool-season grasses. Horse preference for orchardgrass has been inconsistent with some concluding the cool-season grass was highly preferred (Olson et al., 2011; Wilson and Hoormann, 2004), while others concluded it was less preferred by horses (Allen et al., 2013). Although others have determined that Kentucky bluegrass was highly preferred by grazing horses (Allen et al., 2013), the current results were likely impacted by weed invasion. Weed populations in all plots containing Kentucky bluegrass were greater than 50%. However, differences in preference are observed only when a choice is given (Marten et al., 1987). We hypothesize that differences in forage varieties, maturity at the time of grazing, weather conditions, geographic location, and other species offered at the time of grazing all affect horse preference.

Unfortunately, little data exists investigating the preference of legumes under horse grazing compared to other livestock. When evaluating the current data, no legume is consistently highly preferred or less preferred, perhaps because of the relatively low amount of legumes found in each plot (Table 1). Generally, it appears that cool-season grasses mixed with white clover are highly to moderately preferred with $\geq 61\%$ removal. However, white clover was found in the greatest percentage (13-32%) in plots. Cosgrove et al. (1996) also found that heifers selected white clover over ryegrass; however, the extent of selection depended on season. In a recent review, Rutter (2006) detailed 17 studies where sheep, dairy cattle, dairy heifers and beef heifers also preferred white clover. Although little data exists on horse preference of legumes, data is available for other livestock. Norton et al. (1990) determined that the grazing preference of goats were similar to cattle, but different from sheep. Both goats and cattle had a high

preference for legumes during summer and autumn, while sheep had a high preference for grasses in all seasons (Norton et al., 1990).

Table 2. Horse preference and yield of mixed legume grass pastures in 2013.

Forage Specie or Mixture	Horse Preference[1] % Removal	Yield Tons/A
Alfalfa Kentucky bluegrass mixture	38de	0.26^{bcd}
Alfalfa meadow fescue mixture	64^{bc}	1.33^{abc}
Alfalfa orchardgrass mixture	76^{ab}	0.65^{abcd}
Birdsfoot trefoil Kentucky bluegrass mixture	32^{e}	0.19^{cd}
Birdsfoot trefoil meadow fescue mixture	76^{ab}	1.45^{a}
Birdsfoot trefoil orchardgrass mixture	55^{cd}	0.94^{abcd}
White clover Kentucky bluegrass mixture	62^{bc}	0.57^{abcd}
White clover meadow fescue mixture	88^{a}	0.64^{abcd}
White clover orchardgrass mixture	61^{bc}	1.39^{ab}
Kentucky bluegrass	29^{e}	0.10^{cd}
Meadow fescue	64^{bc}	1.22^{abcd}
Orchardgrass	54^{cd}	1.35^{abc}

[1]Within a column, means without a common superscript letter differ ($P \leq 0.05$)

Figure 5. Birdsfoot trefoil meadow fescue mixture pre-grazing

Figure 6. Birdsfoot trefoil meadow fescue mixture post-grazing

Forage yield from July and August of 2013 are listed in Table 2; all yields were corrected for weed percentage. Yield ranged from 1.45 to 0.10 tons/acre on a DM basis and was different among the mixtures and species ($P = 0.0004$). The birdsfoot trefoil and meadow fescue and white clover and orchardgrass mixtures produced the most yield, while the birdsfoot trefoil and Kentucky bluegrass mixture and Kentucky bluegrass seeded alone produced the least amount of yield. However, yields were greatly affected by weed pressure. The top yielding mixtures had $\leq$ 25% weeds, while the lowest yielding forage had $\geq$ 88% weeds. Greater yields would be expected with season-long harvests (May through October) and with better stands of forage. Allen et al. (2012) determined that under horse grazing, orchardgrass produced the highest season long yield with $\geq$ 4.5 tons/acre, while creeping foxtail, smooth bromegrass, and timothy produced the lowest season long yield with $\leq$ 3.9 tons/acre.

Forage nutritive values are listed in Table 3. The amount of CP ranged from 20 to 25%, and was only different among three mixtures ($P = 0.0011$). The mixture of birdsfoot trefoil and meadow fescues had a greater amount of CP compared to Kentucky bluegrass and the alfalfa Kentucky bluegrass mixture. However, CP is rarely limiting in a pasture setting. Adult horses at maintenance require approximately 12% CP (NRC, 2007); all species and mixtures contained greater than 12% CP.

ADF ranged from 24 to 34%, while NDF ranged from 36 to 62% (Table 3). Kentucky bluegrass had the greatest amount of ADF, while the white clover and Kentucky bluegrass mixture had the lowest amount ($P < 0.0001$). Kentucky bluegrass mixed with birdsfoot trefoil, alfalfa and while clover had the lowest amount of NDF, while Kentucky bluegrass planted alone had the greatest amount ($P < 0.0001$). The addition of the legume to the mixture reduced ADF and NDF levels for Kentucky bluegrass. ADF and NDF levels of meadow fescue and orchardgrass were not affected by the addition of the legumes. This is possibly due to the low proportion of Kentucky bluegrass found in the mixtures (Table 1). Although no recommendations for the amount of ADF and NDF in the horse diet exist, it is commonly accepted that horse quality hay should contain no more than 45 and 65% ADF and NDF, respectively. Amounts of ADF and NDF in the pasture species and mixtures were below these recommendations.

NSC ranged from 7 to 21% (Table 3) and was different among mixtures and species ($P < 0.0001$). The alfalfa Kentucky bluegrass mixture had the greatest amount of NSC with 21%, while the birdsfoot trefoil and orchardgrass and white clover and orchardgrass mixtures and Kentucky bluegrass and orchardgrass had the least amount of NSC with $\leq$ 8%. However, these results do not agree with previous research. Martinson et al. (2012) and Pettetier et al. (2010) both concluded that cool-season grasses had greater amounts of NSC compared to legumes. This is due to how these species produce and store NSC. For example, alfalfa stores carbohydrates as starch, compared to most cool season grasses (and a few broadleaf species) that store carbohydrates as fructan (Longland and Byrd, 2006; Martinson et al., 2012; Pettetier et al., 2010). It is unclear why the current study resulted in different NSC contents than expected.

Although no recommendations for the amount of NSC in the healthy adult horse diet exist, recommendations do exist for diseased horses. Nonstructural carbohydrate content has been shown to affect horses diagnosed with laminitis and Polysaccaride Storage Myopathy (PSSM). Muscle pain in horses diagnosed with PSSM was exacerbated by feeding diets high in NSC

(Firshman et al., 2003), and Borgia et al. (2009) recommended hay containing ≤10% NSC should be fed to horses affected by PSSM. Frank (2009) recommended a maximum of 12% NSC for the total diet for obese, laminitic and insulin-resistant horses. Several of the mixtures contained NSC amounts that exceeded recommendations for diseased horses.

The recommended Ca:P for adult horses ranges from 2:1 to 6:1 (NRC, 2007). Ca:P were different among mixtures and species and ranged from 3:1 to 1:2 (Table 3; $P < 0.0001$). It is well understood that legumes contain more Ca compared to cool season grasses. This normally results in a Ca:P for legume forages that exceed 3:1, while cool season grasses have Ca:P that are closer to 2:1. This trend was not consistently observed in the current study, likely due to the low amount of legumes found in each mixture. Legumes never exceed 32% of the mixture (Table 1). The inverted Ca:P in many of mixtures and species was not expected. Inverted Ca:P can cause issues with bone development and strength, especially in young, growing horses (NRC, 2007). It is unclear why the current study resulted in inverted Ca:P.

Equine DE was different amount species and mixtures ($P < 0.0001$) and ranged from 1.2 to 0.93 Mcal/lb. For an adult horse in light work, DE intake should be approximately 20 Mcal per day (NRC, 2007). Therefore, most adult horses in light work would require approximately 20 pounds of pasture DM per day to meet their caloric requirement.

Table 3. Forage nutritive value of mixed legume grass pastures in 2013.

	CP[1]	ADF[1]	NDF[1]	NSC[1]	Ca[1]	P[1]	Equine DE[1]
Forage Specie or Mixture	% DM						Mcal/lb
Alfalfa Kentucky bluegrass mixture	20^{b}	28^{bcde}	38^{d}	21^{a}	0.91^{a}	0.34^{ef}	1.18^{a}
Alfalfa meadow fescue mixture	24^{ab}	28^{bcde}	50^{bc}	12^{bcd}	0.38^{bc}	0.41^{bcde}	1.05^{bc}
Alfalfa orchardgrass mixture	22^{ab}	31^{abcd}	57^{ab}	8^{cd}	0.23^{c}	0.43^{bc}	0.99^{cd}
Birdsfoot trefoil Kentucky bluegrass mixture	22^{ab}	27^{cde}	39^{d}	15^{b}	0.94^{a}	0.31^{f}	1.17^{a}
Birdsfoot trefoil meadow fescue mixture	25^{a}	28^{bcde}	51^{bc}	11^{bcd}	0.31^{bc}	0.41^{bcd}	1.04^{bcd}
Birdsfoot trefoil orchardgrass mixture	23^{ab}	31^{abc}	57^{ab}	8^{d}	0.19^{c}	0.47^{ab}	0.99^{cd}
White clover Kentucky bluegrass mixture	24^{ab}	24^{e}	36^{d}	15^{b}	0.97^{a}	0.36^{def}	1.20^{a}
White clover meadow fescue mixture	24^{ab}	26de	44^{cd}	14^{bc}	0.64^{ab}	0.39^{cde}	1.13^{ab}
White clover orchardgrass mixture	24^{ab}	31^{abc}	57^{ab}	7^{d}	0.23^{c}	0.44^{bc}	0.97^{cd}
Kentucky bluegrass	20^{b}	34^{a}	62^{a}	7^{d}	0.22^{c}	0.45^{abc}	0.93^{d}
Meadow fescue	24^{ab}	29^{bcde}	53^{abc}	12^{bcd}	0.22^{c}	0.42^{bcd}	1.02^{bcd}
Orchardgrass	23^{ab}	32^{ab}	60^{ab}	7^{d}	0.22^{c}	0.52^{a}	0.95^{cd}

[1]Within a column, means without a common superscript letter differ ($P \leq 0.05$)

Conclusion

Although weed invasion made it difficult to interpret results, it appears that white clover mixed with Kentucky bluegrass, orchardgrass or meadow fescue may present a viable option for horse owners wanting to plant a grass legume mixed pasture. Future research is necessary to confirm this conclusion and to ensure white clover cool season grass mixtures result in a high yielding, preferred, and nutrient balanced forage sources for grazing horses.

Acknowledgements
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