Diurnal variation in forage nutrient composition and metabolic parameters of horses grazing warm-season, perennial grass-legume mixed pastures

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Abstract. Although warm-season, grass-legume mixed pastures have improved nutritive value and may reduce negative environmental impacts relative to nitrogen-fertilized grass monocultures, no study has been done to evaluate their effect on diurnal variation of non-structural carbohydrates (NSC) and other nutrients, and on the metabolic responses observed in horses' blood and fecal samples. This 2-yr study aimed to investigate the circadian variation in nutrient composition and the fecal and blood metabolic responses in horses grazing these pastures. Forage, fecal, and blood samples were collected every 28 days at 0600, 1200, 1800, and 0000 h, in two years, for measurement of diurnal variation in forage nutrient composition and fecal and blood metabolites. Forage nutrient composition was affected by time of the day, with digestible energy (DE) and NSC increasing at 1800 h, crude protein decreasing after 1200 h and the fiber components increasing at 1200 h. Fecal lactate and blood insulin were also affected by time of the day. Fecal lactate increased from 0600 to 1200 h. Insulin levels were greater at 1800 than at 0600 h. The increased insulin level followed the increased concentration of NSC in the forage. In conclusion, warm-season, grass-legume mixed pastures show a diurnal pattern in forage nutrient composition, with increased NSC later in the afternoon. However, the metabolic responses observed in this study were not sufficient to predispose horses to metabolic dysregulation. The results also indicate that restricting grazing to the morning may reduce the forage nutritive value, with decreased concentration of DE and increased concentrations of the fiber components, which may decrease diet digestibility.

Introduction

The concentration of non-structural carbohydrates (NSC) in forages grazed by horses is important due to the risk of metabolic disorders when NSC are consumed in excess. For horses with metabolic disorders, such as insulin resistance and laminitis, a threshold of 10 to 12% of NSC is recommended (Frank et al. 2010; Borgia et al. 2009). Forages vary in composition diurnally, usually having greater NSC concentration in the afternoon and evening (Kagan et al. 2018). Although there is a similar circadian pattern among forages for concentrations of NSC and other nutrients, warm-season grasses and legumes are typically lower in NSC compared with cool-season grasses (Bailey et al. 2004; Chatterton et al. 1989). Warm-season perennial grasses make up more than 80% of the pastures of horse operations in Florida, with bahiagrass (*Paspalum notatum* Flügge) as the primary forage species grown (Chambliss and Sollenberger 1991; Vasco et al. 2020). In addition to lesser NSC concentrations, warm-season grasses are characterized by generally lesser nutritive value than legumes and cool-season grasses (Coleman et al. 2004). Incorporating legumes into warm-season grass pastures is an alternative to improve nutritive value and reduce the need for nitrogen fertilizer. Among the candidate legumes in the southeastern USA, rhizoma peanut (*Arachis glabrata* Benth.) combines high nutritive value and long-term persistence under various environmental conditions and management practices (Ortega-S et al. 1992; Muir et al. 2011). To our knowledge, no study has been done to evaluate the effect of warm-season, grass-legume mixed pastures on diurnal variation of forage NSC and other nutrients, and on the metabolic responses observable in horses' blood and fecal samples. We hypothesized that warm-season, grass-legume mixed pastures would show diurnal variation in NSC and other nutrients; however, due their low NSC concentration, these variations would cause little to no effect on metabolic responses in horses.

Methods and Study Site

A two-year grazing experiment was conducted for 84 days each year, from July to October 2019 and 2020, at the University of Florida Beef Research Unit, located in Gainesville, FL. Treatments consisted of well-established ‘Argentine’ bahiagrass (BG) monoculture fertilized with N at 120 kg N/ha (BG-N), bahiagrass monoculture with no N applied (BG-No N), and bahiagrass intercropped with ‘Florigraze’ rhizoma peanut (RP) and fertilized with N at 30 kg N/ha (RP-BG). Experimental units were 1 ha and were continuously stocked by two mature horses (534 ± 47 kg body weight) each. Treatments were replicated two times in a randomized complete block design. Forage, fecal, and blood samples were collected every 28 days at 0600, 1200, 1800, and 0000 h, in both years. To measure diurnal variation in forage nutrient composition, forage samples were hand-plucked to represent the animal diet and sent to a commercial laboratory (Equi-Analytical, Ithaca, NY) for chemical analysis. At the same time, fecal samples were collected directly from the rectum and immediately processed for acquisition of fecal fluid, which was immediately measured for fecal pH. An additional aliquot was separated for measurement of fecal lactate. Blood samples were collected by venipuncture of the jugular vein and quantified for glucose, insulin, and plasma urea nitrogen (PUN). Data were submitted to analysis of variance using the GLIMMIX procedure of SAS. Pasture treatment, evaluation day, time of the day, and their interactions were included in the model as fixed effects, while year, block, and interactions with block were included as random effects.

Results and Discussion

Diurnal Variation in Nutrient Composition

Except for starch, diurnal variation in forage nutrient composition was affected by time of the day (Table 1). Digestible energy at 1800 h was greater (P=0.01) than at 0600 h and 1200 h. Crude protein (CP), however, decreased (P=0.01) from 0600 to 1200 h, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) decreased (P=0.02 and P=0.03, respectively) from 1200 to 1800 h. Non-structural carbohydrates increased (P<0.01) over time from 0600 to 1800. Morning grazing is often recommended as a way to limit NSC intake for metabolically challenged horses due to the previously observed lesser NSC early in the day (Kagan et al. 2018). Our data support this conclusion. However, it is important to highlight that while morning grazing reduces intake of NSC, the overall nutritive value of pasture forages is reduced in the morning, as shown here by reduced DE and increased fiber components. In the current study, regardless of the time, NSC concentrations were below the maximum recommended for metabolically challenged horses (Borgia et al. 2009; Frank et al. 2010), which indicates that warm-season, grass-legume mixed pastures may be a safe alternative for those horses.

Table 1. Diurnal variation of nutrient composition pooled across pastures of monoculture bahiagrass (with or without nitrogen) or intercropped with rhizoma peanut and continuously stocked by horses.

	Time of the day (h)					
Item, %	0600	1200	1800	0000	SEM	P value
Digestible Energy, Mcal/kg DM	1.93^{b}	1.92^{b}	1.98^{a}	1.94^{ab}	0.021	0.001
Crude Protein	12.7^{a}	11.8^{b}	11.8^{b}	11.9^{b}	0.62	0.005
Neutral Detergent Fiber	68.8^{ab}	70.1^{a}	68.2^{b}	69.0^{ab}	0.46	0.023
Acid Detergent Fiber	40.2^{ab}	40.3^{a}	39.3^{b}	40.2^{ab}	0.86	0.025
Starch	0.6	0.7	0.9	0.7	0.32	0.087
Non-structural Carbohydrates	4.3^{c}	5.3^{b}	6.2^{a}	5.6^{ab}	0.30	< 0.001

[abc]Means without a common letter within row are significantly different by the PDIFF procedure adjusted by Tukey at the 5% level of significance.

Forage nutrient composition was affected by treatment (Table 2). The RP-BG mixture resulted in the greatest (P<0.01) DE, CP, starch, and NSC concentrations, and the least NDF and ADF levels. The BG

No-N treatment, however, resulted in the least (P<0.01) DE, CP, and NSC, and the greatest NDF and ADF levels. Although RP-BG resulted in the greatest NSC levels, it was still under the threshold of 10-12% NSC recommended for metabolically challenged horses (Borgia et al. 2009; Frank et al. 2010).

Table 2. Nutrient composition of pastures pooled across times of the day for monoculture bahiagrass [with (BG-N) or without N fertilizer (BG-No N)] or intercropped with rhizoma peanut (RP-BG) and continuously stocked by horses.

	Treatment				
Item, %	BG-N	RP-BG	BG-No N	SEM	P-value
Digestible Energy, Mcal/kg DM	1.92^b	2.05^a	1.85^c	0.020	< 0.01
Crude Protein	12.1^b	13.6^a	10.3^c	0.62	< 0.01
Neutral Detergent Fiber	70.5^b	64.3^c	72.2^a	0.41	< 0.01
Acid Detergent Fiber	39.9^b	38.7^c	41.4^a	0.85	< 0.01
Starch	0.6^b	1.0^a	0.6^b	0.32	< 0.01
Non-structural Carbohydrates	5.4^b	5.9^a	4.8^c	0.28	< 0.01

[abc]Means without a common letter within row are significantly different by the PDIFF procedure adjusted by Tukey at the 5% level of significance.

Fecal and Blood Metabolites

Of all fecal and blood metabolites measured, only fecal lactate and blood insulin were affected by time of the day (Table 3). Fecal lactate at 1200 was greater (P=0.02) than at 0600 h. Insulin at 1800 was greater (P=0.04) than at 0600. The increase in insulin levels at 1800 h followed the increase in pasture NSC at the same time, suggesting that the increased NSC induced an insulinemic response in horses. Insulin levels reported in this study are comparable to past research, which averaged 10.9 µIU/mL in grazing horses (McIntosh et al. 2007). Regardless of the peak in insulin at 1800 h, all levels measured in this study were within the adequate range (<62 µIU/mL) for healthy horses (McGowan et al. 2004). Glucose and PUN did not change over time and averaged 79.9 mM and 17.4 mg/dL, respectively.

Table 3. Diurnal variation of fecal and blood metabolites of horses continuously stocked in pastures of monoculture bahiagrass (with or without nitrogen fertilizer) or intercropped with rhizoma peanut.

	Time of the day (h)					
	0600	1200	1800	0000	SEM	*P* value
Fecal Metabolites						
pH	6.78	6.74	6.73	6.78	0.08	0.34
Lactate, mM	1.6^b	2.2^a	2.1^{ab}	1.8^{ab}	0.39	0.02
Blood Metabolites						
Glucose, mM	79.7	77.1	79.7	82.9	6.97	0.55
Insulin, µIU/mL	6.7^b	6.8^{ab}	8.1^a	7.1^{ab}	0.56	0.04
Plasma Urea Nitrogen, mg/dL	17.6	17.3	17.3	17.2	1.08	0.86

[abc]Means without a common letter within row are significantly different by the PDIFF procedure adjusted by Tukey at the 5% level of significance.

From all fecal and blood metabolites, only insulin, fecal pH, and fecal lactate were affected by pasture treatment (Table 4). The BG-N treatment resulted in fecal pH greater (P=0.04) than BG-No N, while RP-BG fecal pH was intermediate and not different from either of the BG monocultures. Approximately 95% of the volatile fatty acids produced in the hindgut are passively absorbed, and their absorption rate decreases with increasing pH (Argenzio et al. 1975). In the current study, greater fecal pH in horses grazing BG-N compared with BG-No N may suggest that volatile fatty acids were absorbed to a greater extent for those grazing BG-N. Fecal lactate was least (P<0.01) for both RP-BG and BG-No N, while RP-BG and BG-N resulted in increased (P=0.01) insulin levels when compared with BG-No N. For horses with insulin dysregulation and laminitis, forages resulting in lower insulinemic responses can serve as a tool for feeding management (McGowan et al. 2004). Although BG-N and RP-BG resulted in increased insulin levels, these values were within the normal range for healthy horses (McGowan et al. 2004). These findings suggest that although inducing insulinemic responses in horses, warm-season, grass-legume mixed pastures seem to impose low to no risk for horses with metabolic disorders.

Table 4. Fecal and blood metabolites of horses continuously stocked in pastures of monoculture bahiagrass [with (BG-N) or without (BG-No N)] or intercropped with rhizoma peanut (RP-BG) pooled across time of the day.

	Pasture treatment				
	BG-N	RP-BG	BG-No N	SEM	*P* value
Fecal Metabolites					
pH	6.81[a]	6.76[ab]	6.70[b]	0.756	0.04
Lactate, mM	4.1[b]	5.2[a]	5.6[a]	0.47	< 0.01
Blood Metabolites					
Glucose, mM	79.1	80.8	79.6	6.83	0.89
Insulin, µIU/mL	7.9[a]	7.8[a]	5.8[b]	0.71	0.01
Plasma Urea Nitrogen, mg/dL	17.7	18.9	15.4	1.37	0.06

[abc]Means without a common letter within row are significantly different by the PDIFF procedure adjusted by Tukey at the 5% level of significance.

Conclusions

The results of this study support our hypothesis that warm-season, grass-legume mixed pastures show a diurnal pattern in forage nutrient composition with increased NSC later in the afternoon. Although some changes in metabolic responses were elicited, these were insufficient to predispose horses to metabolic dysregulation. The results also indicate that if the grazing period is restricted to the morning only, the nutritional quality of the forage may be less than later in the day, with decreased concentration of digestible energy and increased concentrations of fiber components, which may decrease diet digestibility.

References

Argenzio, R. A., Miller, N., von Engelhardt, W. 1975. Effect of volatile fatty acids on water and ion absorption from the goat colon. *American Journal of Physiology.*, 229: 997-1002.

Bailey, S. R., Marr, C. M., Elliott, J. 2004. Current research and theories on the pathogenesis of acute laminitis in the horse. *Veterinary Journal.*, 167: 129-142.

Borgia, L., Valberg, S., Watts, K., Pagan, J. 2009. Glycemic/insulemic response to feeding hay with different water-soluble carbohydrate content in healthy and Polysaccharide Storage Myopathy-affected horses. *Journal of Equine Veterinary Science.*, *5*: 355-357.

Chambliss, C. G., Sollenberger, L. E. 1991. Bahiagrass: the foundation of cow-calf nutrition in Florida. Proc. Florida Beef Cattle Short course. Univ. of Florida. 40:74-80.

Chatterton, N. J., Harrison, P.A., Bennett, J.H., Asay, K.H. 1989. Carbohydrate partitioning in 185 accessions of graminae grown under warm and cool temperatures. *Journal of Plant Physiology.*, 134: 169-179.

Coleman, S. W., Moore, J. E., Wilson, J.R. 2004. Quality and utilization. Moser, L. E., Burson, B. L., Sollenberger, L. E. editors, Warm-season (C4) grass monograph, ASA, CSSA, Madison, WI. p. 267-308.

Frank, N., Geor, R.J., Bailey, S.R., Durham, A.E. Johnson, P.J. 2010. Equine metabolic syndrome. *Journal of veterinary internal medicine.*, 24: 467-475.

Kagan, I.A., Lawrence, L.M., Seman, D.H., Prince, K.J., Fowler, A.L. Smith, S.R., 2018. Effects of sampling time, cultivar, and methodology on water-and ethanol-soluble carbohydrate profiles of three cool-season grasses in Central Kentucky. *Journal of equine veterinary science.*, 61: 99-107.

McGowan, C.M., Frost, R., Pfeiffer, D.U. and Neiger, R., 2004. Serum insulin concentrations in horses with equine Cushing's syndrome: response to a cortisol inhibitor and prognostic value. *Equine veterinary journal.*, 36: 295-298.

McIntosh, B., Kronfeld, D., Geor, R., Staniar, W., Longland, A., Gay, L., Ward, D., Harris, P., 2007. Circadian and seasonal fluctuations of glucose and insulin concentrations in grazing horses. In Proc. 20th Equine Science Society Symposium, Equine Science Society, Savoy, IL, p. 100-101.

Muir, J. P., Pitman, W. D., Foster, J. L. 2011. Sustainable, low-input warm-season pasture grass-legume mixes: Mission (nearly) impossible? *Grass and Forage Science.*, 66: 301-452.

Ortega-S., J. A., Sollenberger, L. E., Quesenberry, K. H., Cornell, J. A., Jones Jr., C. S. 1992. Productivity and persistence of rhizoma peanut pastures under different grazing managements. *Agronomy Journal.*, 84: 799-804.

Vasco, A. C. C. M., Dubeux, J. C. B., Wallau, M. O., Wickens, C. L., Warren, L. K. 2020. Characterization of Forage Utilization and Pasture Management Practices on Florida Horse Operations. *Journal of Equine veterinary Science.*, 95:103253.