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Original Research

Glucose and Insulin Response of Horses Grazing Alfalfa, Perennial Cool-Season Grass, and Teff Across Seasons

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ABSTRACT

Elevated nonstructural carbohydrate (NSC) values in pasture forages can cause adverse health effects in some horses (*Equus caballus* L.). The objectives of this study were to determine the impact of different forage species on blood glucose and insulin concentrations of horses throughout the grazing season. Research was conducted in July (summer) and September (fall) in St. Paul, MN. Alfalfa (*Medicago sativa* L.), mixed perennial cool-season grasses (CSG), and teff (*Eragrostis tef* [Zucc.] Trotter) pastures were grazed by six horses (24 ± 2 years) that were randomly assigned to one of three forage types in a replicated Latin-square design. Jugular catheters were inserted 1 hour before the start of grazing and horses had access to pasture each day from 08:00 to 16:00 hours. Jugular venous blood samples were collected from each horse before being turned out (0 hours) and then at 2-hour intervals following turnout. Plasma and serum samples were collected and analyzed for glucose and insulin, respectively. Corresponding forage samples were taken by hand harvest. Seasons were analyzed separately and data were analyzed using the MIXED procedure in SAS with $P \leq .05$. Teff generally had lower ($P \leq .05$) equine digestible energy, crude protein, and NSC compared to the other forages. Differences in peak insulin were observed between horses grazing CSG and teff during the fall grazing ($P \leq .05$). These results suggest grazing teff could lower the glucose and insulin response of some horses.

1. Introduction

Obesity, insulin resistance (IR), laminitis, and Equine Metabolic Syndrome (EMS) are growing concerns in the horse industry. Experts estimate that 19%–40% of the horse population is obese [1–4] and 22%–29% is hyperinsulinemic [5,6]. Aged horses may be at a higher risk for these conditions due to decreased exercise, development of metabolic diseases [7], and larger insulinemic responses, which have the capability to lead to hyperinsulinemia or insulin dysregulation [8,9]. Fortunately, management modifications have helped improve the care of horses diagnosed with these metabolic dysfunctions including restricting access to pasture and feeding a high-fiber, low–nonstructural carbohydrate (NSC) diet [10].

Regardless of their horse's disease status, many owners desire pasture access for their horses. However, pasture access may have a detrimental impact on a diseased horse's health due to the lower fiber and higher NSC values of many pasture forages compared to the same forages dried in hay [11]. Across much of the United States, cool-season grasses (CSGs) are the primary forage in horse pastures. However, CSGs tend to have greater amounts of NSC compared to warm-season grasses and legumes [12–14]. Although some research is available on the glucose and insulin response of horses grazing a single pasture species [15–17], little information is available on the effect of horses grazing different pasture species and impacts on the glucose and insulin response. While differences in nutritive values among forage species are known, it is unclear if

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these differences will elicit a unique glucose and insulin response in horses. Therefore, this study investigated the glucose and insulin response of horses grazing alfalfa, CSG, and teff throughout the grazing season. The hypothesis was horses consuming CSG would have a higher glucose and insulin response compared to horses grazing teff with intermediary results observed in horses grazing alfalfa.

2. Materials and Methods

All experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

2.1. Horse Management

Six mares (24 ± 2 years) were body condition scored [18] and challenged with an oral sugar test (Table 1) before the start of the study [19,20]. One horse (horse 6) died unexpectedly following the summer grazing and was replaced with another horse (horse 7) for the fall grazing period; the horse's death was not related to the present research.

Horses had *ad libitum* access to water throughout the study and when not grazing, horses were housed in a dry lot and fed mixed hay containing equal parts alfalfa, CSG, and teff at approximately 2.5% bodyweight (BW) split evenly between two daily feedings. Between the two grazing periods, horses grazed CSG or alfalfa pastures during the day and were housed in a dry lot overnight with *ad libitum* access to CSG hay. Horses were also fed a ration balancer (Enrich Plus Ration Balancing Horse Feed, Purina, St. Louis, MO) at 0.1% BW at 17:00 hours each day to ensure all vitamin and mineral requirements were met for adult horses at maintenance [21].

2.2. Experimental Design and Diets

Horses were randomly assigned to three forage types over three days in a 3 × 3 Latin-square design. Forages consisted of alfalfa, CSG (mixture of orchardgrass [*Dactylis glomerata* L.] and Kentucky bluegrass [*Poa pratensis* L.]), and teff. Alfalfa stands were established on May 2014 in a 0.17 ha pasture and CSG pastures were established on August 2009 in a 0.17 ha pasture. A 0.17 ha teff pasture was established in June 2016 and seeded at a rate of 13.5 kg ha^{-1}. The soil was a Waukegan silt loam (fine-silty over skeletal, mixed, superactive, mesic Typic Hapludoll) with a soil pH of 6.6, 18 ppm P, and 85 ppm K, 13 ppm NO_3-N; no fertilization was needed based on soil test results.

Average forage maturity was assessed before grazing. Alfalfa maturity was assessed using the mean stage count method [22], while maturity for CSG and teff was determined using a scale developed by Moore et al. [23]. Alfalfa was grazed at the early bud stage in the summer and the early flower stage in the fall with the average maturity of three and five in the summer and fall, respectively. The CSG pasture was grazed at a late vegetative stage across seasons. Teff was grazed in the stem elongation and inflorescence emergence phase for the summer and fall, respectively. The average grazing height for the forages before turnout was 58, 42, and 55 cm for alfalfa, CSG, and teff, respectively.

All pastures were mowed to 8 cm 3 weeks before the start of each grazing period to allow for an equal regrowth period. Each pasture was then divided into three equal subplots to allow each horse group (n = 2) access to fresh, ungrazed pasture during the period. Each pasture subplot had sufficient forage available that allowed horses to graze *ad libitum* throughout the 8-hour grazing period. During the summer and fall, forages were grazed on July 19, 21, and 23 and September 12, 14, and 16, respectively, from 08:00 to 16:00 hours. Before the start of each grazing event, horses received a 24-hour hay washout consisting of equal amounts of the three forage species followed by a 12-hour fast. Upon completion of blood collection, horses repeated the hay washout and fasting period before switching treatments. Upon completion of grazing each day, manure was removed from the pastures and forages were mowed to 8 cm and allowed to regrow.

2.3. Sampling and Analysis

Indwelling catheters were inserted approximately 1 hour before the start of blood collection using a local anesthetic (2% lidocaine, Lidocaine 20 mg mL^{-1}, VetOne, MWI Animal Health, Boise, ID) blockade. Blood samples were then taken before turnout at 08:00 (0 hours) and 2, 4, 6, and 8 hours post-turnout, at 10:00, 12:00, 14:00, and 16:00 hours, respectively. Serum samples were collected in 9-mL serum-separator tubes (8,881,302,015; Covidien, Minneapolis, MN) and left at room temperature for 45 minutes following collection. Plasma samples were collected in 10-mL tubes with an ethylenediaminetetraacetic acid additive (8,881,311,743; Covidien, Minneapolis, MN) and put on ice immediately after collection. Following blood collection, catheter lines were flushed with 10 mL of heparinized saline (1,000 units of heparin, 200 mL^{-1} of 0.9% saline). Serum and plasma samples were separated by centrifugation at 1,200 × *g* at 4°C for 20 minutes, supernatants were collected, aliquoted, and stored at −80°C for later analysis.

Glucose concentrations were determined in duplicate by a membrane-based glucose oxidase method (YSI 2300 STAT Plus glucose and lactate analyzer; YSI Incorporated Life Sciences, Yellow Springs, OH) using plasma samples. Insulin concentrations were determined in duplicate serum samples using the EMD Millipore Porcine Insulin Specific RIA Kit (PI-12K; EMD Millipore Porcine Insulin Specific RIA Kit; Billerica, MA, USA) previously validated for use in equine serum [24]. Intra-assay and interassay coefficients of variability (CVs) were calculated using pooled equine serum

Table 1
Group, age, breed, body condition score, and insulin values from an oral sugar test at 0 and 90 minutes for horses used in a grazing study in St. Paul, MN, immediately before study initiation.

Horse	Age, Years	Breed	Body Condition Score	Oral Sugar Test	
				Insulin, μIU mL^{-1}	
				0 Minutes	90 Minutes
1	25	Appaloosa	8	19.9	110.0
2	28	Arabian	8	14.7	69.6
3	23	American Quarter Horse	5	17.2	45.5
4	23	American Paint Horse	6	9.0	40.1
5	21	American Paint Horse	5	5.6	21.9
6	26	Thoroughbred	6	7.0	20.9
7	23	American Quarter Horse	6	6.9	9.0