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Original Research

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

Michelle L. DeBoer [a], Marcia R. Hathaway [a], Kerry J. Kuhle [b], Patty Sue D. Weber [c], Amanda S. Reiter [a], Craig C. Sheaffer [d], M. Scott Wells [d], Krishona L. Martinson [a,*]

[a] *Department of Animal Science, University of Minnesota, St. Paul, MN*
[b] *Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN*
[c] *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI*
[d] *Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN*

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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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* Corresponding author at: Krishona L. Martinson, Department of Animal Science, University of Minnesota, 1364 Eckles Avenue, Saint Paul, MN 55108.
E-mail address: krishona@umn.edu (K.L. Martinson).

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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

samples containing low and high concentrations of insulin. Intra-assay and interassay CVs for the low serum sample were 6.1% and 5.8%, respectively and for the high serum sample, the CVs were 7.4% and 8.4%.

Duplicate, representative forage samples were taken from each pasture at 08:00, 10:00, 12:00, 14:00, and 16:00 to correspond to blood samples. Samples were clipped at 8 cm and dried for 24 hours at 60°C. After drying, samples were ground through a 6-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) followed by a 1-mm screen in a Cyclotec (Foss, Eden Prairie, MN). Samples were mixed thoroughly and subsamples were analyzed for forage nutritive value by a commercial forage testing laboratory (Equi-Analytical, Ithaca, NY). Crude protein (CP) was calculated as the percentage of n multiplied by 6.25, a method determined by the Association of Official Agricultural Chemists (method 990.03). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured using filter bag techniques of ANKOM Technology. Starch concentrations were determined using a glucoamylase enzyme and by measuring dextrose in an automated biochemical analyzer (YSI 2700 select biochemistry analyzer, YSI Incorporated, Yellow Springs, OH). Ethanol-soluble carbohydrates (ESCs) and water-soluble carbohydrates (WSCs) were measured using techniques described by Hall et al. [25]. NSCs were mathematically estimated by adding WSCs plus starch. Equine digestible energy (DE) was calculated using an equation developed by Pagan [26].

2.4. Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) as a replicated 3 × 3 Latin-square design. Day and pen were blocking factors (i.e., rows and columns) in the Latin-square design. Each replicate was the experimental unit for forage. Variables analyzed included equine DE, starch, WSC, ESC, NSC, ADF, NDF, and CP. Data were analyzed using a repeated measure design according to methods used by Littel et al. [27]. The model included treatment, day, pen, hour, and treatment × hour.

Individual horses within a forage treatment were the experimental unit for glucose and insulin concentrations. Variables analyzed included baseline, average, and peak values for both glucose and insulin in the horses. Blood samples taken at hour 0 were included in the model as a baseline covariate for peak and average glucose and insulin values. The average blood values were analyzed using a repeated measure design [27]. The model included the baseline covariate as well as treatment, day, pen, hour, and treatment × hour. A square root transformation was used for insulin values to meet analysis of variance assumptions; data were back transformed for presentation. Means are averaged over the sampling days within the season with the least square means of the MIXED procedure (±standard error) and mean separations determined using Tukey's honest significant difference test ($P \leq .05$). Based on the influencing statistics, horse 1 was considered an outlier and was not included in the analysis. Glucose and insulin area under the curve (AUC) were not reported as a result of differences in grazing time across seasons. In addition, blood values did not return to baseline within the 8-hour grazing period. Statistical significance was set at $P \leq .05$ with trends identified at $P \leq .10$.

3. Results

3.1. Environment

Environmental conditions are reported in Table 2. The environmental conditions observed were similar to historical averages for St. Paul, MN.

Table 2
Mean environmental conditions between 08:00 and 16:00 hours in July (summer) and September (fall) in St. Paul, MN, during the 2016 grazing season.

Days[a]	Summer			Fall		
	1	2	3	1	2	3
Mean temperature (°C)	29.5	30.9	24.6	24.2	17.9	21.2
Total precipitation (cm)	0.01	0	0.85	0	0	0
Mean solar radiation (W M^{-2})	58.6	59.7	8.13	44.6	54.6	25.8

Weather data obtained from http://www.dnr.state.mn.us/climate/historical/index.html.
[a] Days 1, 2, and 3 in the summer correspond to July 19, 21, and 23, respectively; days 1, 2, and 3 in the fall correspond to September 12, 14, and 16, respectively.

3.2. Forage Nutritive Value

Differences in forage nutritive values were observed between the different species for both seasons (Table 3). In the summer and fall, alfalfa had the greatest equine DE ($P \leq .01$) with the lowest equine DE values observed in teff. The only difference observed in NSC was during the summer when teff was lower in NSC compared to CSG ($P \leq .01$). In the summer and fall, NDF was highest in teff and lowest in alfalfa ($P \leq .01$) while the ADF was highest in teff and lowest in CSG ($P \leq .01$). In both seasons, CP was highest in alfalfa and lowest in teff.

Changes in NSC were also evaluated throughout the 8-hour grazing period within each season (data not shown). In the summer, NSC values ranged from 9.2% to 10.7% in alfalfa, 9.8% to 12.6% in CSG, and 7.3% to 9.5% in teff. However, no differences were observed between the different time points. In the fall, NSC values ranged from 7.4% to 11.0% in alfalfa, 9.3% to 11.1% in CSG, and 7.5% to 8.2% in teff. While no differences were observed in CSG and teff across the time points, differences in alfalfa NSC were found. Higher NSC content in alfalfa was observed at hour 8 compared to 0, 2, and 4 hours.

3.3. Glycemic and Insulinemic Response

Baseline glucose values ranged from 88 to 89 mg dL^{-1} in the summer and 86 to 87 mg dL^{-1} in the fall with no differences

Table 3
Forage nutritive values (mean ± standard error)[d] on a dry matter basis for alfalfa, cool-season grass, and teff grazed by horses in July (summer) and September (fall) in St. Paul, MN during the 2016 grazing season.

Nutrient[e]	Alfalfa	Cool-Season Grass	Teff
Summer			
DE (Mcal kg^{-1})	2.29 ± 0.01[a]	2.24 ± 0.01[b]	2.00 ± 0.01[c]
Starch (%)	2.6 ± 0.5	0.6 ± 0.5	0.9 ± 0.5
WSC (%)	7.3 ± 0.6	10.6 ± 0.6	7.5 ± 0.6
ESC (%)	6.0 ± 0.5	7.6 ± 0.5	5.1 ± 0.5
NSC (%)	9.8 ± 0.6[ab]	11.3 ± 0.6[a]	8.4 ± 0.6[b]
NDF (%)	46.3 ± 0.2[c]	52.9 ± 0.2[b]	67.0 ± 0.2[a]
ADF (%)	34.8 ± 0.3[a]	30.3 ± 0.3[b]	37.1 ± 0.3[a]
CP (%)	22.5 ± 0.5[a]	24.1 ± 0.5[a]	14.2 ± 0.5[b]
Fall			
DE (Mcal kg^{-1})	2.51 ± 0.02[a]	2.18 ± 0.02[b]	2.03 ± 0.02[b]
Starch (%)	1.8 ± 0.5	0.9 ± 0.5	1.3 ± 0.5
WSC (%)	6.8 ± 0.1[b]	9.1 ± 0.1[a]	6.6 ± 0.1[c]
ESC (%)	5.5 ± 0.4[b]	7.4 ± 0.4[a]	5.4 ± 0.4[b]
NSC (%)	8.6 ± 0.4	9.9 ± 0.4	7.9 ± 0.4
NDF (%)	36.9 ± 0.9[c]	55.4 ± 0.9[b]	63.3 ± 0.9[a]
ADF (%)	28.7 ± 0.2[c]	31.4 ± 0.2[b]	36.8 ± 0.2[a]
CP (%)	27.3 ± 0.7[a]	22.8 ± 0.7[ab]	17.0 ± 0.7[b]

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DE, equine digestible energy; ESC, ethanol-soluble carbohydrates; NDF, neutral detergent fiber; NSC, nonstructural carbohydrate; WSC, water-soluble carbohydrates.
[a,b,c]Within a row, means without a common letter superscript differ based on a Tukey test ($P \leq .05$); means without a superscript were not different ($P > .05$).
[d] Means were averaged within a season over three sampling days.
[e] Measured as percent dry matter.

observed ($P \geq .05$; Table 4). In addition, neither average nor peak glucose was different for horses grazing alfalfa, CSG of teff, regardless of season (Table 4). Baseline insulin values ranged from 7.4 to 8.8 μIU mL^{-1}, with no differences observed ($P \geq .05$; Table 4). No differences were observed in average insulin values or peak insulin values in the summer (Table 4). However, horses grazing teff had lower peak insulin values in the fall when compared to CSG (Table 4, $P \leq .05$). When evaluating differences in the blood characteristics between horses grazing the different forage species at each time point after turnout, no differences were observed (data not shown).

4. Discussion

4.1. Forage Nutritive Value

Nutritive values of feedstuffs are crucial to properly balance horse rations, especially for horses diagnosed with metabolic diseases. Frank [28] suggested a total diet ≤12% NSC for horses diagnosed with EMS, and Borgia et al. [29] recommended hay containing ≤10% NSC for horses affected by polysaccharide storage myopathy. Furthermore, Staniar et al. [16] observed a relationship between sugar and starch and the glycemic and insulinemic responses of horses, Rodiek and Stull [30] observed a relationship between NSC and the glycemic index of horses, and Gordon et al. [31] determined that a low NSC meal would lead to a lower glucose and insulin response. However, age could alter these recommendations as decreased insulin sensitivity has been observed in aged horses [9].

In the present study, CSG contained higher NSC levels in comparison to teff during the summer. This was anticipated because CSG stores excess carbohydrates in the form of fructans that can be translocated to the stem [32]. In comparison, legumes and warm-season grasses have a self-limiting carbohydrate storage mechanism. As a result, lower NSC values are often observed in legumes and warm-season species when compared to CSG species [32]. While minimal research has compared the NSC content of these different forage species, DeBoer et al. [13] found teff pastures averaged ≤9% NSC and Staniar et al. [33] observed NSC contents ranging from 5 to 8% in teff hay. In addition, Rodiek and Jones [34] determined that alfalfa and teff hay had NSC levels ≤12% while oat and wheat hay had NSC values ≥30%. In comparison, cool-season perennial grasses have reported NSC values ranging from 6 to 17% [11] while cool-season annual grasses had NSC values ranging from 10 to 22% [14]. Considering feedstuffs with ≤12% NSCs have been suggested as a low-NSC feed for horses [28,29], based on the present study, both teff and alfalfa would be considered low-NSC feeds. However, variables including maturity, environmental conditions, and management decisions could have an impact on NSC concentrations and should be considered in specific cases and future research.

Diurnal variations can also impact the NSC content of forages with NSC values increasing throughout the day and decreasing overnight [35,36]. While the present study evaluated the NSC content during a typical daytime grazing period, from 08:00 to 16:00 hours, changes in NSC were only observed in alfalfa in the fall. Future research should evaluate impacts of these forages on blood metabolites over a 24-hour period. Another variable that can influence NSC is the preservation of plant materials following cutting. In the present study, plants were subject to oven drying at 60°C for 24 hours before nutritive analysis. Recent studies prefer freeze drying or microwave pretreatment to inactivate hydrolytic enzymes that can alter the carbohydrate values in a plant [37]. As a result, NSC values reported in the present study may be lower than the actual NSC concentrations present in the fresh pasture.

Recent studies have determined that NSC content alone is not a good predictor of glucose and insulin response of horses consuming feedstuffs. In the present study, NSC did not vary across forage species in the fall ($P > .05$); however, variations in peak insulin were observed between horses consuming teff and CSG pastures during that season. Although consistent regrowth periods were given, plant species were at different maturities and heights during grazing. Siciliano et al. [38] found a greater insulinemic response in horses grazing tall versus shorter sward heights. As a result, variations in sward heights due to different forage growth habits could have contributed to different responses observed in the present study.

In addition to considering the role of growth patterns and sward height in glucose and insulin response, Richards and Kempton [17] found that digestibility of NSC played an important role in the glycemic and insulinemic response, and NSC alone was incapable of predicting the response. Teff consistently had higher NDF and ADF values and horses grazing teff had a lower insulinemic response in the fall. Fiber concentrations play an important role in digestibility of a feedstuff and likely influenced the insulin response of grazing horses [17].

Although the nutritive components of a feed are important, the long-term effects of the feedstuff on the physiological response of the horse should also be considered. Because obesity is one of the major contributing factors for horses diagnosed with EMS and IR [11,39], weight management is important. The equine DE content of a feedstuff is commonly associated with BW maintenance, loss, or gain. The equine DE of teff was 0.2 to 0.5 Mcal kg^{-1} lower compared to CSG and alfalfa, respectively. Based on the DE requirements for an adult horse at maintenance [21], a horse consuming a total forage diet at 1.5% BW would lose one body condition score [18] in 2.5 months when consuming teff, or gain one body condition score in 3 months when consuming alfalfa. Horses consuming CSG would maintain their BW. Future research should explore whether teff is able to elicit BW loss in overweight horses over a longer time period.

4.2. Glycemic and Insulinemic Response

In the present study, baseline glucose values ranged from 86 to 89 mg dL^{-1}, which is comparable to values (84–96 mg dL^{-1}) observed in previous research [16,40,41]. The peak glucose values observed in the present study ranged from 94 to 101 mg dL^{-1},

Table 4
Glucose and insulin values (mean ± standard error)[c] of horses (n = 5) grazing alfalfa, cool-season grass, and teff in July (summer) and September (fall) in St. Paul, MN, during the 2016 grazing season.

Blood Characteristics	Alfalfa	Cool-Season Grass	Teff
Summer			
Baseline glucose, mg dL^{-1}	88.2 ± 1.4	89.1 ± 1.4	88.0 ± 1.4
Average glucose, mg dL^{-1}	93.3 ± 1.3	93.7 ± 1.3	95.8 ± 1.2
Peak glucose, mg dL^{-1}	94.4 ± 2.2	100.8 ± 2.3	95.6 ± 2.2
Baseline insulin, μIU mL^{-1}	7.6 ± 1.8	7.6 ± 1.8	7.4 ± 1.8
Average insulin, μIU mL^{-1}	23.3 ± 9.3	26.2 ± 9.9	26.0 ± 9.9
Peak insulin, μIU mL^{-1}	32.3 ± 9.4	39.7 ± 9.5	32.1 ± 9.5
Fall			
Baseline glucose, mg dL^{-1}	86.8 ± 2.0	86.7 ± 1.5	85.8 ± 1.9
Average glucose, mg dL^{-1}	94.8 ± 0.6	92.8 ± 0.5	93.6 ± 0.8
Peak glucose, mg dL^{-1}	99.5 ± 1.5	100.3 ± 1.2	97.2 ± 1.4
Baseline insulin, μIU mL^{-1}	8.6 ± 2.0	8.5 ± 1.6	8.8 ± 1.9
Average insulin, μIU mL^{-1}	41.2 ± 3.9	45.1 ± 3.3	32.7 ± 3.4
Peak insulin, μIU mL^{-1}	53.3 ± 4.1[ab]	60.5 ± 3.2[a]	39.1 ± 3.9[b]

[a, b] Within a row, means without a common letter superscript were identified as trends with a $P \leq .10$; means without a superscript were not different ($P > .10$).
[c] Means reported were averaged within a season over 3 sampling days.

which is also comparable to past research where values ranged from 99 to 104 mg dL^{-1} [16,38].

Baseline insulin values of 7 to 9 μIU mL^{-1} were observed in the present study. These values were slightly lower compared to values of 10.8 and 13.4 μIU mL^{-1} observed by Siciliano et al. [38]. Peak insulin values ranged from 32 to 40 μIU mL^{-1} and 39 to 61 μIU mL^{-1} during the summer and fall grazing periods, respectively. Peak insulin values were greater than those observed by Staniar et al. [16] and Siciliano et al. [38] who observed values ≤43 and 31 μIU mL^{-1}, respectively. However, these differences could be a result of seasonal variations, as peak insulin concentrations were ≤39 μIU mL^{-1} during the summer grazing in the present study. In addition, the previous studies evaluated blood glucose and insulin during continuous grazing while the present study evaluated blood glucose and insulin following a 12-hour fast, which could have contributed to these differences.

Average insulin concentrations while grazing ranged from 23 to 26 uIU mL^{-1} and 33 to 41 uIU mL^{-1} during the summer and fall, respectively. McIntosh et al. [15] observed mean insulin concentrations as low as 10.9 μIU mL^{-1} when evaluating grazing horses across seasons on CSG. The higher insulin response observed in the present study could be a result of differences in horse age [8,9], forage nutritive values [30], management decisions including sward height at the time of grazing [38], use of fertilizer [42], individual horses, and fasting before consumption [43]. However, a limitation to this study is the small sample size used to evaluate the response, which may explain the lack of differences observed. Future research using a larger sample size is necessary to confirm these results.

Furthermore, aged horses, regardless of body condition, have demonstrated decreased insulin sensitivity that can put these horses at a higher risk of becoming hyperinsulinemic or developing insulin dysregulation [8,9]. Jacob et al. [9] found that horse age influenced peak insulin concentrations, baseline insulin, peak insulin, and AUC insulin with higher values observed in aged compared to adult horses. These results suggest age is an important factor when considering glucose and insulin responses in horses. Future research should include different age groups when evaluating glucose and insulin responses from grazing different forages.

5. Conclusions

The results from this study are some of the first to compare the glycemic and insulinemic responses of horses grazing different pasture species across seasons. While no differences were observed in the glucose response of horses grazing different forage species, horses grazing teff had a lower peak insulin response in the fall when compared to horses grazing CSG. While most of the nutritional differences were observed between CSG and teff, the high equine DE concentrations consistently observed in alfalfa suggest it is most suitable for horses with high energy requirements including performance horses or pregnant and lactating mares. The lower insulin value observed in horses grazing teff, combined with the lower equine DE content and nutrient profile, suggests it could be beneficial as a grazing option for horses requiring an attenuated insulinemic response as well as BW loss commonly associated with metabolic issues.

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