

**Equine postprandial glycemic and insulinemic responses to beet pulp
prepared with increasing water concentrations**

Honors Thesis

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1. Introduction

Blood glucose and insulin concentrations are commonly used to assess metabolic regulation in horses [1,2]. Like humans, horses benefit from diets that regulate glucose and insulin levels. The objective of this study was to evaluate the effect of graded water-to-beet pulp ratios on postprandial (i.e., after feeding) glycemic and insulinemic responses in non-insulin dysregulated horses. The hypothesis was that increasing water concentration during beet pulp preparation would prolong meal consumption time and moderate postprandial glycemic and insulinemic responses. Previous work has evaluated glycemic responses to beet pulp prepared using different methods [3]. This earlier study showed that beet pulp preparation could be assessed in relation to postprandial response, but graded water concentrations and insulin responses were not assessed [3]. Re-examining beet pulp preparation may help inform practical feeding recommendations for horses.

Beet pulp shreds are a byproduct of sugar beet processing and are commonly used as a fibrous feed in equine and other livestock diets [4,5]. During processing, most sucrose is removed to produce crystallized sugar and molasses, leaving beet pulp as the fibrous byproduct, along with some residual sugars [5,6]. Beet pulp contains soluble fiber, particularly pectin, that can be fermented in the hindgut and used to produce energy via volatile fatty acids [7,8]. This makes it a common feed for performance horses and senior horses to increase fiber intake [9,10].

Although dry beet pulp can be safely consumed, horse owners often soak it before feeding. Soaking changes the texture, increases water inclusion, and may improve palatability [11,12]. Adding water to feed increases the meal volume and could change feeding behavior and rate of consumption, and possibly postprandial metabolic responses [12,13].

Following a meal, nonstructural carbohydrates (NSC), including sugars, starch, and fructans, are digested and absorbed in the small intestine, increasing blood glucose concentrations and stimulating pancreatic insulin secretion [14,15]. Insulin binds to insulin receptors on target tissues, activating intracellular signaling pathways that stimulate glucose transporters to move glucose into the cell [15,16]. A postprandial increase in blood glucose is expected, and a moderate insulin response supports normal glucose uptake [17,18].

Horses differ in the degree of their insulin response, and excessive or prolonged elevations in circulating insulin concentrations are of greater concern [19,20]. This pattern, known as insulin dysregulation (ID), is a key characteristic of equine metabolic syndrome (EMS) and occurs commonly with obesity [21,22]. The resulting hyperinsulinemia directly influences an increased risk of endocrinopathic laminitis, laminitis associated with metabolic dysfunction, rather than systemic inflammation or trauma [23,24].

Assessing how feeds influence postprandial glucose and insulin responses is relevant even in non-insulin dysregulated (NID) horses, as feed composition can alter metabolic responses [25,26]. Horse feeds contain variable proportions of nonstructural carbohydrates (NSCs) and structural fiber [27]. NSCs are digested and rapidly absorbed in the small intestine, whereas fiber is fermented in the hindgut to produce volatile fatty acids [14]. Nutritional management strategies for horses at risk of metabolic disorders emphasize limiting NSCs while increasing reliance on forage and other fiber-based feeds [21,28].

2. Materials and Methods

2.1 Ethical Approval

All procedures were approved by the Colorado State University Institutional Animal Care and Use Committee (Protocol #6350, approved date 10/21/2024).

2.2 Horses

Four horses housed at the Colorado State University Equine Teaching and Research Center (Fort Collins, CO, USA) were used in this study. Two horses, a Warmblood gelding and Warmblood mare, were from the CSU English riding program. Two horses, an Appendix gelding and a Thoroughbred gelding, were from the CSU Polo Team. The horses had a mean age of 13.5 ± 1.94 years and a mean body weight (BW) of 576.4 ± 30.6 kg at the start of the study. A review of Colorado State University's Veterinary Teaching Hospital records cited no prior diagnosis of insulin dysregulation or laminitis.

Table 1

Horses used in the study

Horse	Breed	Sex	Age (yr)	Initial Body Weight (kg)
H1	Appendix	Gelding	12	583.0
H2	Thoroughbred	Gelding	16	531.6
H3	Warmblood	Mare	14	592.4
H4	Warmblood	Gelding	12	598.7

The horses were maintained in dry lot pens, fed free-choice grass/alfalfa hay, and had free access to water. The horses also received 453.6 g (1 lb.) ration balancer (Purina Enrich Plus®, Purina Animal Nutrition, Gray Summit, MO, USA) once daily at 16:00, as part of their basal diet. Horses were maintained at a moderate workload as defined by NRC (2007) throughout the study.

2.3 Experimental Design & Beet Pulp Treatments

A 4 × 4 Latin square crossover design was used to evaluate four beet pulp preparation treatments with increasing water-to-beet pulp ratios. The four horses received each treatment once over four study periods, with the treatment order assigned using a standard Latin square design.

All treatments contained 453.6 g (1 lb.) of beet pulp per meal.ⁱ The beet pulp was Plain Dried Beet Pulp Shreds (Unbeetable Feeds®, San Rafael, CA, USA) with a guaranteed analysis of 6.0% minimum crude protein, 0.30% minimum crude fat, 22.0% maximum crude fiber, 15.0% maximum moisture, and 10.5% maximum nonstructural carbohydrates (NSC). Independent analysis by Dairy One Forage Laboratory (Ithaca, NY, USA) indicated a beet pulp moisture content of 7.4%, 42.8% non-fiber carbohydrates (NFC), 41.0% neutral detergent fiber (aNDF), 24.9% acid detergent fiber (ADF), 8.7% crude protein, 1.0% crude fat, and 6.56% ash.

Beet pulp was portioned using a bench scale and placed into individual 10-quart buckets. Treatments were prepared daily according to assigned water-to-beet pulp ratios. Tap water (20 °C) was weighed and added to the beet pulp. The feed was lightly mixed, and the shreds were fully covered with water. Treatments were prepared at 16:00 before feeding, allowing for a 14-hour overnight soak. Beet pulp meals were provided at 08:00, as horses were moved from their group pens to a dry lot pen for individual feeding and monitoring.

The study was conducted over eight weeks during early spring. Each study period included 9 days of adaptation to the assigned treatment, followed by a postprandial blood sampling day. Horses then returned to the basal diet for a 4-day hay-only washout before the next period.

Table 2.

Treatment schedule for the 4 × 4 Latin square design

	10 day	4 day wash out	10 day	4 day wash out	10 day	4 day wash out	10 day	4 day wash out
Horse 1	T1 Dry		T2 2:1		T3 4:1		T4 6:1	
Horse 2	T2 2:1		T1 Dry		T4 6:1		T3 4:1	
Horse 3	T3 4:1		T4 6:1		T1 Dry		T2 2:1	
Horse 4	T4 6:1		T3 4:1		T2 2:1		T1 Dry	

2.4 Postprandial Blood Sampling

On Day 10 of each period, horses had access to hay overnight and were removed from their pens at 06:00. At 07:00, jugular vein catheters (14-gauge, 4-inch; MILA) were aseptically placed following subcutaneous administration of 1 mL lidocaine at the insertion site. Catheters were placed 60 minutes prior to feeding, and baseline blood samples were collected 30 min after placement.

After catheter placement, horses were led to individual 24 × 20-ft dry lot pens without access to feed but with access to automatic waterers. At 08:00, the horses were fed their assigned beet pulp treatment for that period. Observers recorded the time the horse began eating until the horse finished the meal or refused to consume more.

Blood samples (10 mL) were collected via the jugular catheter into vacutainers at baseline (0 min) and 15, 30, 60, 90, 120, 180, 240, 300, and 360 min postprandially. Catheters were flushed with heparinized saline between collections. Whole blood glucose was measured immediately after collection using a handheld glucometer (AlphaTrak 3 Blood Glucose Monitoring System, Zoetis®, Kalamazoo, MI, USA), calibrated for horses and reporting glucose concentration in mg/dL from approximately 0.3 μ L of whole blood.

Blood collected into the vacutainer was centrifuged for plasma separation, and the plasma was transferred and stored at -20 °C until analysis. Plasma insulin was analyzed at 0, 30, 60, 90, 180, and 240 min by radioimmunoassay at the Animal Health Diagnostic Center Endocrinology Laboratory (Cornell University College of Veterinary Medicine, Ithaca, NY, USA). Insulin was reported in μ IU/mL. Postprandial insulin area under the curve (AUC) was calculated by trapezoidal numerical integration between consecutive sampling time points [29].

Postprandial insulin area under the curve (AUC) was calculated by trapezoidal numerical integration between consecutive sampling time points

2.5 Statistical Analysis

Data were analyzed using a mixed-model ANOVA in SAS® v9.4 (Cary, NC). Fixed effects included horse, treatment, time, and the treatment \times time interaction when significant. Periods were included as a random effect. Significant effects were compared using least squares means (LSMeans). Data are presented as mean \pm standard error of the mean with statistical significance declared at $P \leq 0.05$.

3. Results

3.1 Feed Waste

On an as-fed basis, feed waste differed by treatment ($p = 0.040$), but not by horse ($p = 0.29$). On a dry matter basis, feed waste did not differ among treatments or horses ($p \geq 0.12$).

Table 3

Feed waste (dry matter basis) by treatment and horse

Treatment	Feed waste (g)	Horse	Feed waste (g)
T1 (0:1)	36.4 ± 19.73^a	H1	76.275 ± 19.73^a
T2 (2:1)	35.825 ± 19.73^a	H2	83.7 ± 19.73^a
T3 (4:1)	70.375 ± 19.73^a	H3	28.675 ± 19.73^a
T4 (6:1)	69.6 ± 19.73^a	H4	23.55 ± 19.73^a

Note. Values represent least squares means \pm SEM.

3.2 Consumption Time

Consumption time differed by treatment ($p = 0.005$) and horse ($p = 0.005$). T1 had the shortest consumption time (11.8 ± 1.9 min), while T4 had the longest (24.0 ± 1.9 min). T2 and T3 were intermediate. H1 took the longest to consume the meal (24.7 ± 1.9 min), while H3 and H4 were shorter. H2 was intermediate.

Table 4

Consumption time (min) by treatment and horse

Treatment	Consumption time (min)	Horse	Consumption time (min)
T1 (0:1)	11.8 ± 1.9^a	H1	24.7 ± 1.9^a
T2 (2:1)	14.7 ± 1.9^{ab}	H2	19.2 ± 1.9^{ab}
T3 (4:1)	20.7 ± 1.9^{bc}	H3	15.7 ± 1.9^b
T4 (6:1)	24.0 ± 1.9^c	H4	11.5 ± 1.9^b

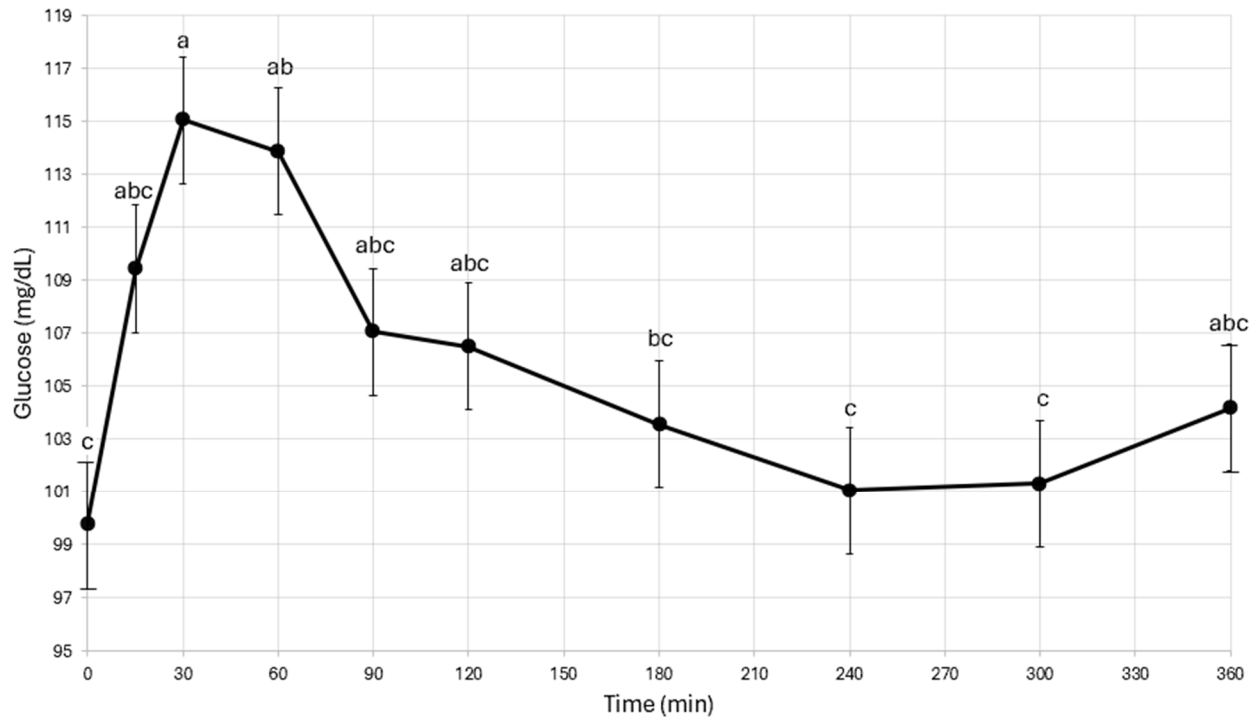
Note. Values are least squares means \pm SEM. Means with different superscripts differ ($p < 0.05$).

3.3 Postprandial Glycemic Response

Blood glucose concentrations (mg/dL) were affected by time ($p < 0.001$) and horse ($p < 0.001$), with no effect of treatment ($p = 0.908$) and no treatment \times time interaction ($p = 0.097$). Blood glucose rose from baseline (0 min; 99.75 ± 2.4 mg/dL), peaking at 30 min postprandially (115.06 ± 2.4 mg/dL), and returning toward baseline by 90 min (107.06 ± 2.4 mg/dL).

Figure 1.

Postprandial blood glucose concentrations (mg/dL), pooled across water-to-beet pulp treatments



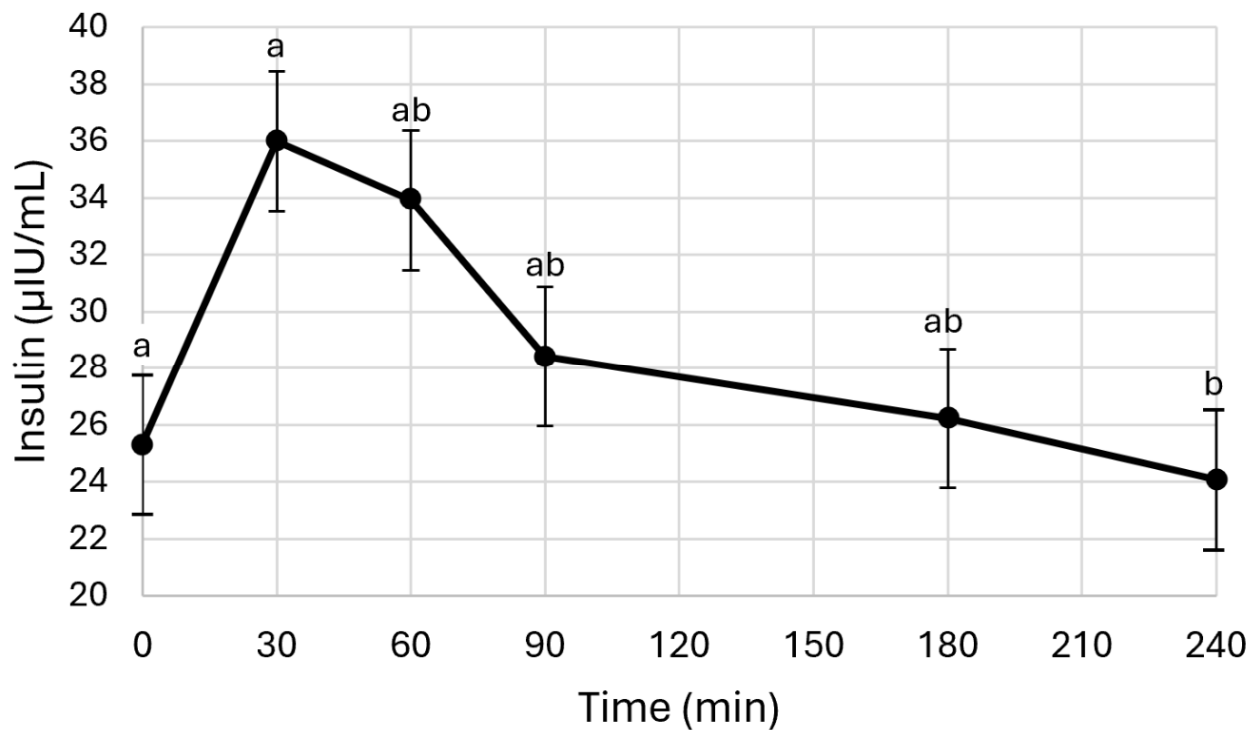
Note. Values represent least squares means (LSMeans \pm SEM). Means with different superscripts differ over time ($p < 0.05$).

3.4 Postprandial Insulinemic Response

Plasma insulin concentrations ($\mu\text{IU/mL}$) were affected by time ($p = 0.003$) and horse ($p < 0.001$), with no effect of treatment ($p = 0.40$) and no treatment \times time interaction ($p = 0.99$). Insulin concentrations increased from baseline (0 min; $25.30 \pm 2.45 \mu\text{IU/mL}$) peaking at 30 min postprandially ($36.00 \pm 2.45 \mu\text{IU/mL}$) and declined by 60 min ($33.92 \pm 2.45 \mu\text{IU/mL}$) toward baseline, with further decreases at 90 min ($28.42 \pm 2.45 \mu\text{IU/mL}$), 180 min ($26.24 \pm 2.45 \mu\text{IU/mL}$), and 240 min ($24.07 \pm 2.45 \mu\text{IU/mL}$).

Figure 2.

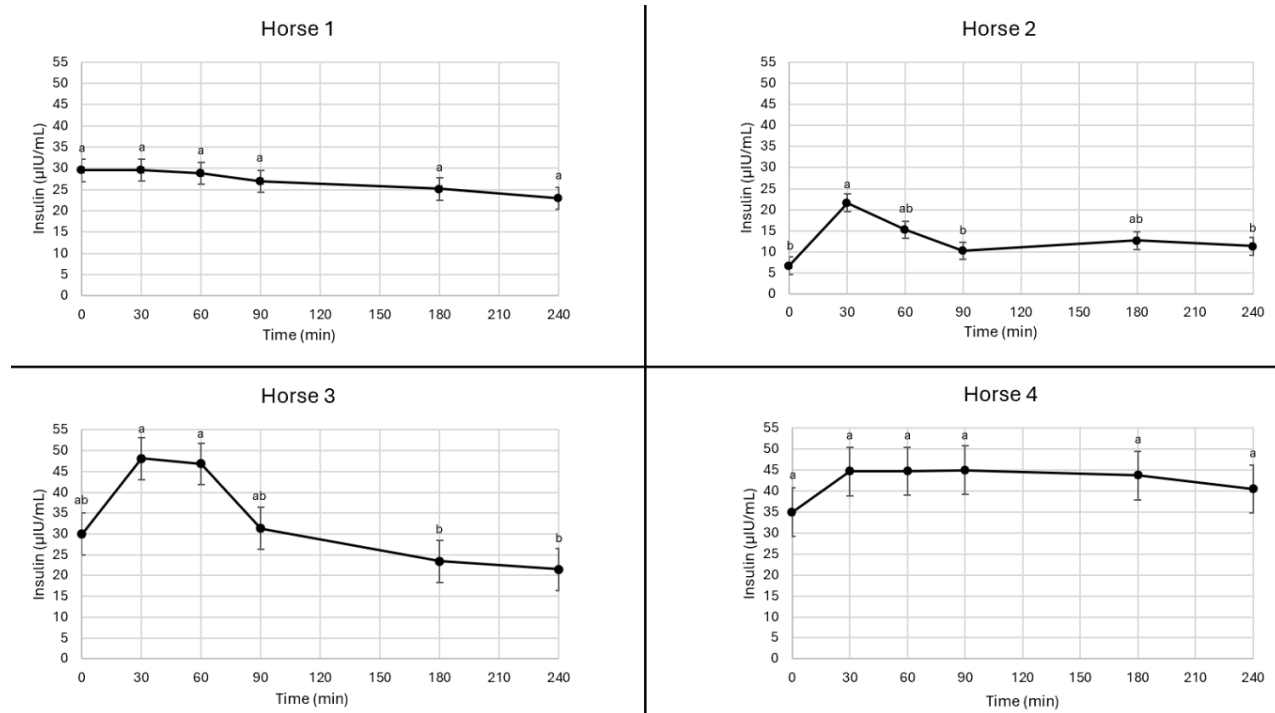
Postprandial plasma insulin concentrations ($\mu\text{IU/mL}$), pooled across water-to-beet pulp treatments



Note. Values represent least squares means ($\text{LSMeans} \pm \text{SEM}$). Means with different superscripts differ over time ($p < 0.05$).

Figure 3.

Postprandial plasma insulin concentrations ($\mu\text{IU/mL}$) over time for individual horses pooled across water-to-beet pulp treatments.



Note. Values represent least squares means (LSMeans \pm SEM). Means with different superscripts differ over time ($p < 0.05$).

3.5 Insulin Area under the Curve

Total insulin AUC (0-240 min) was calculated using the trapezoidal method based on insulin concentrations measured at 0, 30, 60, 90, 180, and 240 min [29]. Total insulin area under the curve (AUC; 0–240 min) was not affected by treatment ($p = 0.775$) but was affected by horse ($p = 0.0016$). Insulin AUC was lowest in H2 ($3116.44 \pm 865.18 \mu\text{IU/mL}\cdot\text{min}$) and highest in H4 (10401.19 ± 865.18).

Table 5*Insulin area under the curve (AUC) by treatment and horse*

Treatment	Insulin AUC (μIU/mL·min)	Horse	Insulin AUC (μIU/mL·min)
T1 (0:1)	6302.33 \pm 865.18 ^a	H1	6393.34 \pm 865.18 ^{bc}
T2 (2:1)	6577.16 \pm 865.18 ^a	H2	3116.44 \pm 865.18 ^c
T3 (4:1)	7463.1 \pm 865.18 ^a	H3	7580.74 \pm 865.18 ^{ab}
T4 (6:1)	7149.11 \pm 865.18 ^a	H4	10401.19 \pm 865.18 ^a

Note. Values represent least squares means (LSMeans \pm SEM). Means with different superscripts differ ($p < 0.05$).

4. Discussion

4.1 Feed Waste and Dry Matter Intake

Dry matter intake was comparable across treatments, as shown in Table 3, as it is the NRC-recommended approach for evaluating feed with different moisture concentrations [27]. This agrees with Grimm et al., who reported that soaking beet pulp did not alter dry matter intake when comparing soaked to dry beet pulp [12].

Water-to-beet pulp ratios were based on preliminary testing with Unbeetable beet pulp shreds soaked overnight in room-temperature tap water, where all assigned water volumes were absorbed before feeding. This reflects a more practical barn setting than Humer et al., who used a saliva-like solution to evaluate feedstuff absorption and expansion related to choke risk [30]. Although dry matter waste did not differ among horses, numerical differences suggest that individual eating behavior likely influenced orts and dropped feed. Intake rate and feed waste can vary among horses and across feeding setups [31,32].

4.2 Consumption Time and Intake Behavior

Similarly, Grimm et al. reported that horses, when fed soaked beet pulp, slowed their intake rate compared to when they were fed dry beet pulp [12].

In the present study, it was observed that when the horses consumed T3 and T4, they appeared to have longer eating bouts, periods of continuous intake without lifting the head [32]. In a forage context, Glatter et al. reported that soaked and steamed hay were consumed more slowly than dry hay, supporting the idea that the physical effect of water on feed can impact intake behavior [33]. During preliminary testing, horses were excluded because they refused to consume dry beet pulp, suggesting individual variation in palatability.

Horse-to-horse variation was evident; H1 took more than twice as long to consume the treatments (24.7 ± 1.9 min) as H4 (11.5 ± 1.9 min). Observed eating behaviors also differed, as H4 remained directly in front of his bucket and rarely lifted his head.

Dry beet pulp raises owner concerns regarding esophageal obstruction, or choke. Humer et al. evaluated beet pulp expansion in a saliva-like solution and reported a high choke factor [30]. In contrast, *Equine Applied and Clinical Nutrition* states that dry beet pulp is not inherently associated with choke [11]. There were no incidences of esophageal obstruction in the present study. Choke risk may correlate more to individual feeding behavior, such as rapid intake, than to beet pulp.

4.3 Post-Meal Blood Glucose Response

Blood glucose levels changed over time after feeding and also varied among individual horses. This response is expected because glucose levels normally rise after a meal as carbohydrates are digested and absorbed into the bloodstream [14].

This pattern of a temporary rise followed by a return toward baseline is typical after beet pulp consumption and has been reported in previous studies [[3,26].

Research by Rodiek and Stull demonstrated that beet pulp produces a lower glycemic response than feeds that are high in rapidly available carbohydrates, confirming its role as a high-fiber feed with a relatively low impact on blood glucose [34]. In the present study, the amount of water added to beet pulp did not affect blood glucose levels. This finding suggests that increasing water content changed the physical form of the meal but did not change how much glucose was absorbed or how much carbohydrate was consumed. This finding is also consistent with the similar amounts of feed waste observed across treatments, indicating that horses ate comparable amounts of beet pulp dry matter regardless of water level. Because dry matter intake was similar, nutrient intake was also likely similar. Beet pulp used in this study had a relatively low non-structural carbohydrate (NSC) content and was high in fiber, further supporting its classification as a low-glycemic feed.

Water itself does not provide energy, so differences in blood glucose would only be expected if soaking changed carbohydrate availability, digestion rate, or total nutrient intake. Since the beet pulp and soaking water were fully consumed in this study, those factors remained consistent across treatments. Previous studies using a 4 x 4 Latin square design reported changes in glycemic response due to altering beet pulp composition, such as adding molasses, rinsing away soluble sugars, or discarding soaking water [3,26,35]. In contrast, this study maintained the full beet pulp–water mixture, meaning nutrients were not added or removed. As a result, water inclusion alone did not alter glucose response.

Karasu et al. reported that glucose and insulin responses differ depending on the feeding sequence and on whether meals are fiber- or starch-enriched, underscoring the importance of

standardized feeding conditions in the present study [36]. The present study retained the full beet pulp-water treatment meal, so the nutrients provided remained available for consumption. A small increase in glucose was observed several hours after feeding (between 240 and 360 minutes). This later rise may be related to the fermentation of beet pulp fiber in the hindgut, which produces volatile fatty acids that can later contribute to glucose production [37]. Although these fermentation products were not measured, this explanation is consistent with the known high-fiber nature of beet pulp [34].

4.4 Post-Meal Insulin Response

Plasma insulin levels also changed over time after feeding and differed among horses. This response is expected, as insulin is released when blood glucose rises following carbohydrate digestion [38]. Insulin helps move glucose from the bloodstream into tissues.

This pattern closely matches previously reported insulin responses following beet pulp consumption [26]. The parallel curves over time shown in Figures 1 and 2 suggest coordinated postprandial glucose regulation and insulin secretion following beet pulp consumption.

Importantly, the amount of water added to the beet pulp did not affect insulin response. This finding aligns with the lack of treatment effects on blood glucose levels and dry matter intake, suggesting that horses consumed similar nutrients across all treatments.

Sampling continued through 240 minutes after feeding, allowing both the insulin peak and the return toward baseline to be captured. Previous research shows that feeds higher in starch produce larger insulin responses, but beet pulp contains relatively low amounts of starch and sugar [2,25,39,40]. The beet pulp used in this study had a maximum non-structural carbohydrate (NSC)

value of 10.5%, limiting the stimulus for a strong insulin response. The finding that insulin-dysregulated horses had greater postprandial insulin responses than non-insulin-dysregulated horses supports that metabolic phenotype can influence insulin response to NSC intake [39].

For context, insulin concentrations above 60 $\mu\text{IU/mL}$ at 60 or 90 minutes are often used to indicate abnormal responses in oral sugar tests. Although this cutoff is not designed for beet pulp feeding, it provides a reference point showing that insulin responses in this study were modest and well within a normal range [1].

4.5 Individual Insulin Dynamics

Insulin AUC provided a way to evaluate insulin dynamics by capturing total insulin exposure across the sampling period [16]. Insulin responses varied among individual horses despite similar health status and management.ⁱⁱ The findings support the importance of evaluating individual insulin responses in horses, particularly because insulin dysregulation is a central feature of equine metabolic syndrome and likely with multiple contributing factors [21,28].

Insulin AUC provided a way to evaluate insulin dynamics by capturing total insulin exposure across the sampling period [16]. Insulin responses varied among individual horses despite similar health status and management.ⁱⁱⁱ The findings support the importance of evaluating individual insulin responses in horses, particularly because insulin dysregulation is a central feature of equine metabolic syndrome and likely with multiple contributing factors [21,28].

Differences in total insulin exposure may expose distinctions in insulin sensitivity, pancreatic responsiveness, incretin signaling, or insulin clearance relative to glucose concentration [38,41]. Bamford et al. reported breed-related differences in insulin sensitivity and insulinemic responses to oral glucose in horses and ponies of moderate body condition score [19]. De Laat et

al. further described that the exaggerated insulin secretion response to oral carbohydrates that marks insulin dysregulation may also involve the enteroinsular axis, including incretin signaling and the gut-pancreas connection [41]. Individual variation in postprandial metabolism is also described in humans, where responses to the same meal can differ substantially among individuals [42].

5. Conclusion

Increasing the water-to-beet pulp ratio did not change postprandial glucose or insulin levels. Glucose and insulin were influenced only by time: they rose after the horses ate, peaked, and then returned toward baseline, which is exactly what we expect in healthy horses. The horses exhibited individual variation in both baseline values and overall responses, likely reflecting differences in insulin sensitivity, as is also seen in humans. More research is needed to understand how different beet pulp preparation methods affect horses with insulin dysregulation.

As more water was added, horses took longer to eat and produced more as-fed waste, but dry-matter waste stayed the same. This shows that the extra “waste” was just water, not actual differences in beet pulp intake. Overall, increasing water-to-beet pulp ratios may change how horses eat, but it does not appear to affect their glucose or insulin responses. These findings support beet pulp without molasses as a low-glycemic fiber source that can be prepared in different ways to match horse preferences and management needs.

6. My Future Direction

I first found interest in metabolism in Equine Nutrition when I took Dr. Hess’s class. I completed the Honors option for that course by writing a review on Equine Metabolic Syndrome.

Later, during a summer internship, Dr. Catalano and Dr. Hess suggested a project looking at how horses' blood sugar responds to beet pulp.

I am incredibly grateful for all the guidance and support my Honors committee gave me, from collecting data to helping me practice my presentation. Their commitment has shaped my entire undergraduate experience at Colorado State University.

This fall, I shall start my master's program at Texas A&M with Dr. Erica Macon. I intend to build on this project by sharpening the research question and adding more depth. While writing this manuscript, I realized there is no clear information on how much water beet pulp actually absorbs before it stops soaking in. A future study could rethink the treatment levels. Instead of four water levels, I want to compare dry beet pulp, a moderate amount of added water, and the maximum amount of water beet pulp can hold while still being palatable to horses.

One limitation of my study was that every horse received exactly one pound of beet pulp. In the future, it would make more sense to adjust the amount based on body weight (g/kg BW) to better compare digestion and metabolic responses. Another challenge was getting a true baseline blood sample. From working with the horses daily at the Equine Teaching and Research Center, I suspect stress from catheter placement, weather, and being separated from the herd affected their baseline values. They did calm down eventually, so future studies should allow more time between catheter placement and baseline sampling.

For future blood sampling, Dr. Macon suggested starting the 15-minute timer after the horse finishes eating, not when the feed is offered. This would better reflect the actual metabolic response, especially since wetter beet pulp took longer to eat. Extending sampling beyond six hours, at a lower frequency, could also help identify whether there is a secondary rise in glucose, possibly from gluconeogenesis. Including horses with insulin dysregulation would let us evaluate

whether soaked beet pulp is still a safe fiber source for them. And measuring responses to the kinds of meals owners actually feed their animals would make the results more practical.

This study can be repeated. I am extremely thankful that I had the opportunity, both this time and in the future, with such supportive advisors, great friends, and inspiring institutions.

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ⁱⁱ Treatment 1 (T1) consisted of dry beet pulp (0:1 water: beet pulp). Treatment 2 (T2) was moist beet pulp prepared at a 2:1 water-to-beet pulp ratio (907.2 g water). Treatment 3 (T3) was damp beet pulp prepared at a 4:1 ratio (1,814.4 g water). Treatment 4 (T4) was soaked beet pulp prepared at a 6:1 ratio (2,721.6 g water). Horses underwent a brief palatability trial before the study to confirm acceptance of all treatments.

ⁱⁱ Horse affected both plasma insulin concentrations ($p < 0.001$) and insulin AUC ($p = 0.0016$), with H2 having the lowest AUC ($3116.44 \pm 865.18 \mu\text{IU}/\text{mL} \cdot \text{min}$) and H4 the highest AUC (10401.19 ± 865.18), while H1 (6393.34 ± 865.18) and H3 (7580.74 ± 865.18) were intermediate.

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