

The Effect of Feeding Frequency on Blood Cortisol Concentrations in Horses

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Introduction

- The horse industry relies largely on high starch concentrates to meet the energy needs of performance horses
- Deviations from normal feeding patterns can negatively impact behavior
- Horses will graze intermittently in a pasture setting, consuming small amounts of forage frequently throughout the day
- Horses will not interrupt feed intake for more than 4 hours (Baumgartner et al., 2020).
- Cortisol is a glucocorticoid hormone that serves as a mediator of the stress response and gluconeogenesis
- High cortisol levels increases the rate of gluconeogenesis
- Cortisol concentrations increase during periods of stress and can be used to identify causes of persistent stress in horses
- Cortisol levels follow circadian rhythm and are highest from 6:00 AM to 12:00 PM and lowest from 4:00 PM to 12:00 AM (Widmann, 2010).
- Indications of stress around feeding frequency could impact the way horses are fed

Objective

- To determine how manipulating feeding frequency impacts stress in horses.

Study Design

- The feed trial and blood samples were collected at Ohio State University and the serum samples were processed at Virginia Tech
- 12 stock breed horses from Ohio State University's equestrian program
- 3x2 Latin square design for treatment
- Manipulation of feeding frequency
 - All horses housed outside, except for last 24 hours of the trial period
 - Horses had access to hay that was split into two meals at 1.55% of body weight
 - Cortisol measurements based on feeding a high starch concentrate
 - Fed at 0.45% of body weight
 - Treatment groups consisted of 1, 2, and 3 meals daily
 - All horses were subject to each treatment in a different order
 - Blood was collected during the first feeding for each group
 - 6 blood samples collected for each horse from 0 minutes to 180 minutes after the concentrate was provided

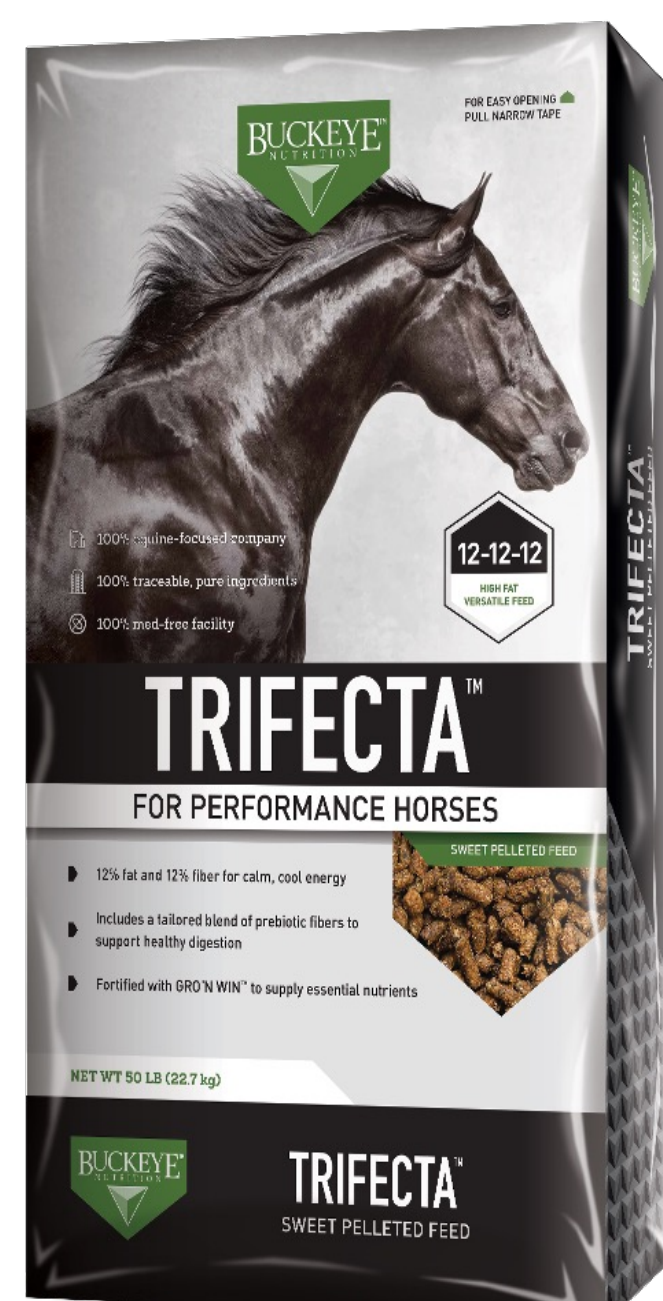


Figure 1: The selected concentrate

GUARANTEED ANALYSIS

Ingredient	Amount
Crude Protein, Min.	12.00%
Lysine, Min.	0.85%
Methionine, Min.	0.20%
Threonine, Min.	0.55%
Crude Fat, Min.	12.00%
Crude Fiber, Max.	12.00%
Acid Detergent Fiber, Max.	17.00%
Neutral Detergent Fiber, Max.	30.00%
*Starch, Max.	12.00%
*Sugar, Max.	8.00%
Calcium, Min.	1.00%
Calcium, Max.	1.50%
Phosphorus, Min.	0.50%
Potassium, Min.	0.90%
Copper, Min.	55 ppm
Zinc, Min.	225 ppm
Selenium, Min.	0.60 ppm
Vitamin A, Min.	7,000 IU/lb
Vitamin D, Minimum	700 IU/lb
Vitamin E, Min.	200 IU/lb
Omega-6 Fatty Acids, Min.	6.00%
Omega-3 Fatty Acids, Min.	1.00%
Saccharomyces cerevisiae, Min.	0.90%

Analyzing Cortisol Concentrations

- Serum processed through DRG Cortisol ELISA
- Cortisol levels were determined for each horse from $t_0 - t_{180}$ within the three treatment groups
- Average cortisol levels for each time interval were determined for comparison amongst the treatment groups



Figure 2: 96 well plate for plate reader assay and Multi-Channel Pipette

Cortisol Concentration Data

Table 1: The average cortisol levels for all 12 horses as a result of manipulating feeding frequency

	Frequency		
Time (minutes)	1x	2x	3x
0	136.1725	162.746	126.6254
30	137.8191	145.9137	120.5786
60	158.4949	155.1397	142.0461
90	143.3838	124.6732	115.6483
120	133.3506	122.826	110.1638
180	116.2833	124.1841	106.0418

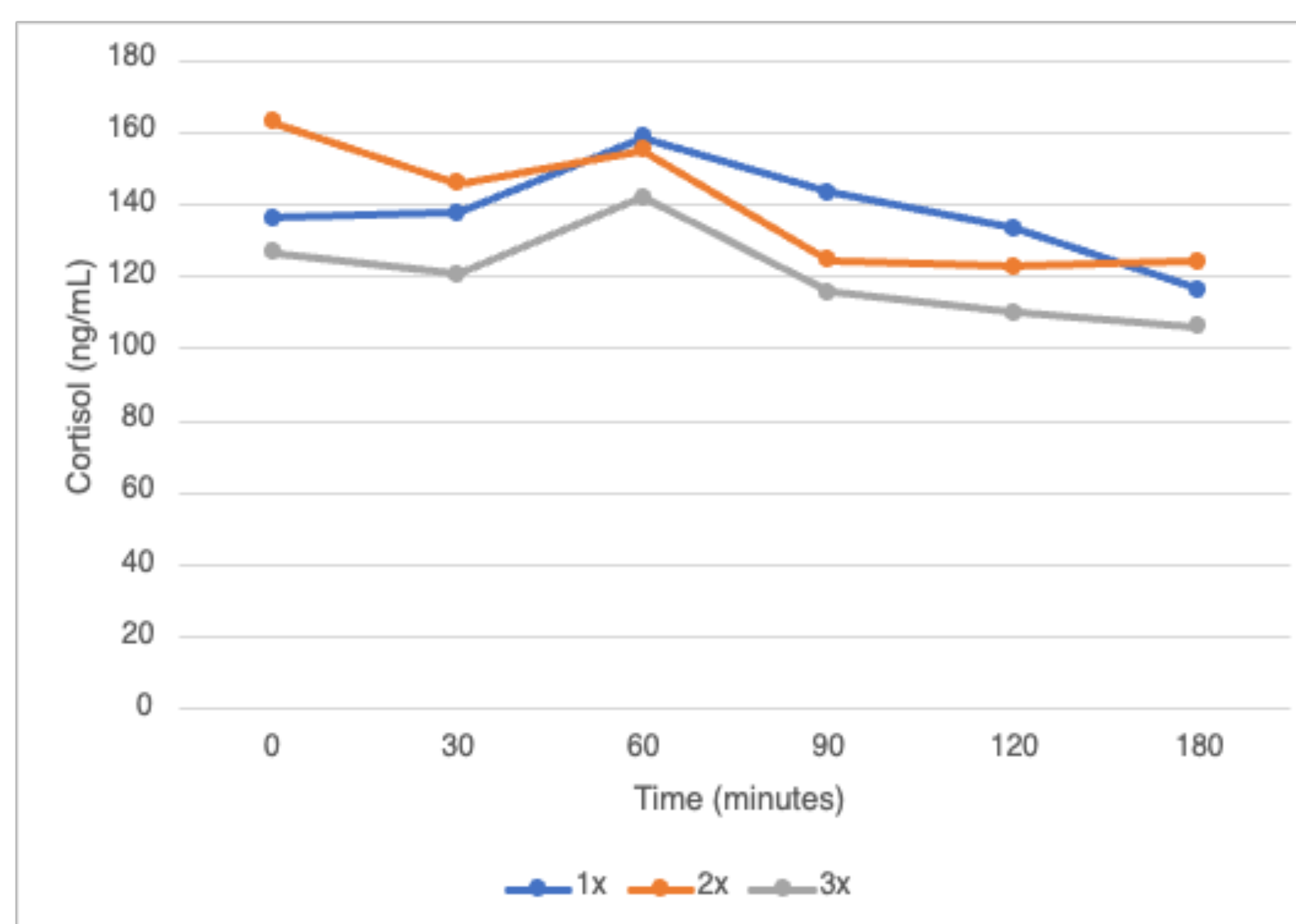


Figure 2: Cortisol concentrations for the three treatment groups as a result of the elapsed time after the concentrate had been provided

Results

- Spike in cortisol levels 60 minutes after eating
- The group fed two meals had the highest initial cortisol levels, but they leveled off after t_{60}
- 1 meal per day had a sustained increase in cortisol levels after 60 minutes
- 3 meals per day resulted in the lowest overall cortisol levels
- There was little fluctuation in cortisol concentrations from t_0 to t_{180} amongst the three treatment groups

Discussion

- Despite the similarities in cortisol concentrations amongst the three treatment groups, the group that had 3 meals per day showed the lowest overall serum cortisol concentrations, suggesting that horses fed smaller meals throughout the day were less stressed
- Horses fed only one concentrate meal per day had the highest sustained cortisol concentrations, providing evidence that deviating from normal feeding behavior is a possible stressor for horses
- Cortisol is a regulator of gluconeogenesis, meaning that the higher levels of cortisol observed with the group fed once daily could be a result of compensation for fewer starch rich meals, rather than stress
- Cortisol levels follow circadian rhythm and are highest in the morning
- This study did not evaluate the cortisol concentrations during the afternoon and evening feedings, so more research needs to be done to determine if the increase in cortisol concentrations are a result of stress or normal fluctuations in cortisol levels based on the time of day.
- Feeding horses three smaller concentrate meals best reflects their normal propensity to consume small, frequent meals
- Given that the group fed three times per day had the lowest overall cortisol concentrations, the industry's feeding practices should be re-evaluated and modified to better match horses' natural feeding patterns

Literature Cited

Baumgartner, Miriam, et al. "Common Feeding Practices Pose a Risk to the Welfare of Horses When Kept on Non-Edible Bedding." *Animals*, vol. 10, no. 3, 2 Mar. 2020, <https://doi.org/10.3390/ani10030411>.

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